PHENOGENETIC STUDIES ON SCUTE-1 OF DROSOPHILA MELANOGASTER.

I. THE ASSOCIATIONS BETWEEN THE BRISTLES AND THE EFFECTS OF GENETIC MODIFIERS AND TEMPERATURE

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Received March 16, 1934

INTRODUCTION

This paper deals with the quantitative variations, under different environmental conditions, of the bristle numbers of the mutant scute-1 in *Drosophila melanogaster*. The genetic factor "scute" was discovered by BRIDGES in 1916 (unpublished). Since 1929, when other alleles of scute were found at the same locus, the original scute has been designated as scute-1. Figure 1 shows the position and nomenclature of the bristles

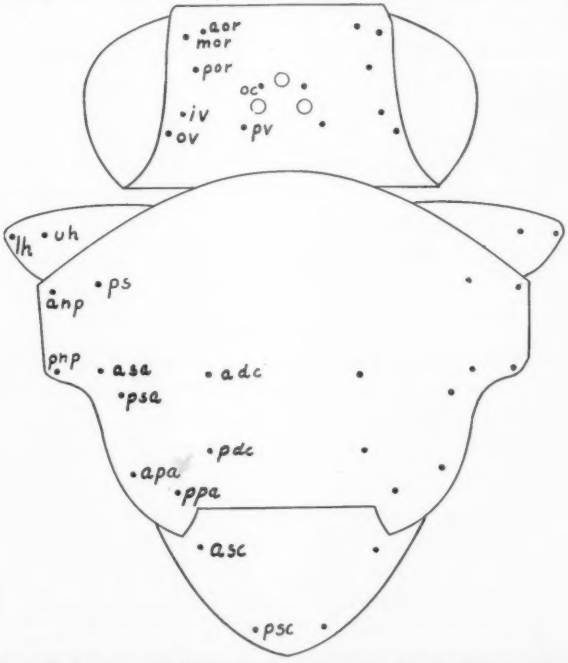

FIGURE 1.—(After PLUNKETT 1926) Location and names of bristles of *D. melanogaster* (wild-type): aor, mor, por, anterior, middle, and posterior orbitals; oc, ocellar; pv, postvertical; iv, ov-inner and outer verticals; u h, l h, upper and lower humerals; anp, pnp, anterior and posterior notopleurals; ps, presutural; asa, psa, anterior and posterior supra-alars; a pa, p pa, anterior and posterior postalars; adc, pdc, anterior and posterior dorsocentrals; a sc, p sc, anterior and posterior scutellars.

of the wild-type fly, and the abbreviations used throughout this paper. Scute-1 flies differ from wild in the absence of certain of the bristles in a certain proportion of the flies. The proportion of flies in which these bristles are missing is affected by genetic and environmental factors. The mean number (in a particular group of flies) of each bristle is susceptible to exact quantitative determination. These means have been determined under various definite environmental conditions in stocks homozygous for genetic modifiers, to obtain evidence on the effects of certain environmental and genetic modifying factors in conjunction with effect of the "main" genetic factor scute-1.

The present paper is concerned with the following questions:

1. Does scute affect the same bristle under different conditions, specifically at different temperatures?
2. Are all the bristles affected in the same way by modifying factors, specifically (a) genetic modifiers, (b) temperature?
3. Do the several bristles vary independently of each other, or are there definite associations between them?

ACKNOWLEDGMENTS

I wish to express my deepest gratitude to Dr. C. R. PLUNKETT for his aid and guidance and to my colleagues for their sympathetic help during the elaboration of this work.

EXPERIMENTAL

Materials and methods

Culture methods

The usual Drosophila culture methods were employed with additional precautions for insuring uniform conditions (PLUNKETT 1926). The food medium in each half pint milk bottle consisted of 30 gm water to 30 gm ripe banana with two percent of agar-agar to gel the mixture. The bananas were never boiled and the food never used until twenty-four hours after being made up. Where only fifty offspring were expected per bottle, two drops of a thick yeast suspension, (one cake to 100 cc water) were added after the gel had set. It was found during the course of the experiments that no effects of crowding were obtained when as many as 300 flies were raised in a bottle to which solid crumbled yeast was added from time to time as the larvae required it. The temperature was regulated in incubators held constant to within 0.1°.

Flies four to seven days old, raised under favorable conditions, were used for egg laying. Twenty pairs were used in each bottle for laying periods of one-half to three hours depending on the number of offspring desired.

Selection

Before starting the experiments the scute-1 flies were inbred for 22 generations (brother-sister matings) and selected for low bristle numbers in order to make them uniform for all genetic factors affecting bristle numbers.

Table 1 shows the parent-offspring and left-right correlation coefficients for the bristles counted.

TABLE 1

Correlation coefficients.

BRISTLE	TEMP.	$r_{♀♀}$	$r_{♂♂}$
A. PARENT-OFFSPRING			
aor+mor	28°	+0.12 ±0.070	−0.02 ±0.060
oc	28°	−0.13 ±0.070	−0.092±0.61
anp	28°	+0.06 ±0.073	−0.02 ±0.060
asc	15°	+0.10 ±0.13	+0.14 ±0.12
psc	15°	+0.19 ±0.12	
B. LEFT-RIGHT			
aor	28°	+0.05 ±0.037	−0.02 ±0.038
mor	28°	+0.04 ±0.036	+0.02 ±0.038
oc	28°	+0.09 ±0.036	+0.04 ±0.038
anp	28°	+0.02 ±0.036	−0.004±0.038
asc	15°	+0.02 ±0.041	+0.04 ±0.041
psc	15°	+0.002±0.041	+0.06 ±0.041
pv	15°	+0.04 ±0.041	−0.04 ±0.041
apa	15°	+0.18 ±0.079	+0.35 ±0.088
adc	28°	+0.22 ±0.069	+0.12 ±0.060
iv	14°		+0.54 ±0.11

The parent-offspring correlations are not statistically significant, and of themselves would indicate the success of the inbreeding in making the stock uniform for modifiers affecting the bristles being selected. The use of the left-right correlation coefficients as a measure of genetic and environmental uniformity has been described by PLUNKETT (1926). The frequency of occurrence of a bristle on either side of any fly depends upon the genetic factors present in the fly and the environmental factors acting upon it. In an isogenic stock raised in a uniform environment, the bristles should be distributed at random among the flies. If such is the case the frequency of flies with two, one and zero bristles should be p^2, $2p(1-p)$ and $(1-p)^2$ respectively, p being the mean number of any particular bristle per half fly (PLUNKETT 1926). In other words there should be no correlation between the two sides of a fly. The absence of any left-right correlation (table 1) for most of the bristles shows that this condition has been realized in this test. The stock is therefore suitable for quantitative phenogenetic studies. This also justifies using the "half fly" rather than the whole fly

as the unit. The statistically significant positive left-right correlations obtained for the adc, apa and iv bristles indicate that the stock has not been made fully isogenic for genetic modifiers of these bristles.

The result shows that some genetic modifiers, at least, do not affect all the bristles similarly. This will be discussed more fully later (p. 119).

The associations between bristles

To ascertain whether or not the several bristles affected by scute vary independently of each other, the degrees of association between the bristles on the same side, as measured by the association coefficients (YULE 1911) were determined. Let A indicate the frequency of presence, that is, number of half flies having the bristle, and a the frequency of absence of a particular bristle and let B and b indicate the frequency of presence and absence, respectively, of another particular bristle. The association between these bristles is measured by

$$\frac{(AB)\ (ab)-(Ab)\ (aB)}{(AB)\ (ab)+(Ab)\ (aB)}$$

where AB is the number of half flies having both bristles, ab the number having neither bristle, et cetera. There is complete positive association (association coefficient $=+1$) between the bristles when either

$$(AB)=A\ \therefore\ (Ab)=0\ \text{and}\ (ab)=b$$
$$\text{or}\ (ab)=a\ \therefore\ (aB)=0\ \text{and}\ (AB)=B.$$

There is complete negative association between the bristles (association coefficient $=-1$) when either

$$(AB)=0$$
$$\text{or}\ (ab)=0.$$

There is no association between the bristles (association coefficient $=0$) when

$$\frac{(AB)}{B}=\frac{(Ab)}{b}$$

and therefore

$$(AB)\ (ab)=(Ab)\ (aB).$$

The statistical significance of the association coefficient can be obtained by the following method. If there is no association

$$\frac{(AB)}{B}=\frac{(Ab)}{b}=\frac{A}{N}$$

where N is the total number of half flies in the population. The proportion

of A in the population is therefore the theoretical value that $\frac{(AB)}{B}$ and $\frac{(Ab)}{b}$ should each have if the bristles were unassociated. The standard error of the difference between $\frac{(AB)}{B}$ and $\frac{(Ab)}{b}$ is therefore

$$\sqrt{\frac{A}{N}\left(1-\frac{A}{N}\right)\left(\frac{1}{B}+\frac{1}{b}\right)}.$$

If there is an association between the bristles the difference between $\frac{(AB)}{B}$ and $\frac{(Ab)}{b}$ should be greater than twice its standard error.

In table 2 the association coefficients are listed, with the differences between $\frac{(AB)}{B}$ and $\frac{(Ab)}{b}$ and the standard errors of these differences.

TABLE 2

Associations between bristles.

TEMP.	BRISTLES	♀♀ ASSOC. COEF.	♀♀ $\frac{(AB)-(Ab)}{B \quad b}$ S.E.	♂♂ ASSOC. COEF.	♂♂ $\frac{(AB)-(Ab)}{B \quad b}$ S.E.
28°	aor–mor	−1.0	$\frac{0.195}{0.026}$	−1.0	$\frac{0.17}{0.024}$
28°	oc–anp	+0.047	$\frac{0.025}{0.025}$	−0.057	$\frac{0.011}{0.024}$
28°	mor–oc	+0.014	$\frac{0.013}{0.025}$	+0.053	$\frac{0.019}{0.035}$
28°	mor–anp	+0.049	$\frac{0.025}{0.045}$	+0.030	$\frac{0.005}{0.030}$
28°	anp–asc	+0.014	$\frac{0.004}{0.029}$	+0.040	$\frac{.011}{0.014}$
28°	mor–asc	−0.038	$\frac{0.008}{0.029}$	−0.12	$\frac{0.002}{0.030}$
28°	oc–asc	+0.057	$\frac{0.012}{0.029}$	−0.12	$\frac{0.005}{0.030}$
14°	apa–asc	−0.14	$\frac{0.07}{0.015}$	−0.19	$\frac{0.021}{0.047}$
14°	iv–mor			−0.33	$\frac{0.13}{0.15}$
15°	asc–psc	−0.25	$\frac{0.09}{0.057}$	+0.42	$\frac{0.024}{0.038}$

A clearly significant association is found only between the aor and mor bristles. This complete negative association suggests that these two bristles, which I had been considering as different, are possibly the same bristle shifted in position. The aor in the wild fly is much longer and closer to the median line than the mor, and directed anteriorly, whereas the mor is directed posteriorly. In the scute-1 flies the aor is small, seldom in the same position as in the wild-type and pointing in various directions in different flies. A comparable shifting of the pdc bristles in Dichaete has been observed by PLUNKETT (1926). In scute-3, which removes aor and mor bristles in definite proportions of the flies, these two bristles have been found on the same side and in the same relation to one another as in the wild-type. What I have called the "aor" in scute-1 may, then, in all probability be a shifted mor. Because of the difficulty in distinguishing between these two bristles, they were recorded jointly in many of the experiments.

The absence of association between any of the other bristles is evidence that they vary independently of each other. In a later section the absence of association will be discussed with reference to the concept of a pattern of bristle removal which has been postulated for scute by a number of investigators.

Effects of temperature

Effect on mean bristle numbers

Flies were reared at various temperatures from 14° to 31° for their total period of development, to determine the mean bristle numbers at each temperature. Most of the means are based on counts of at least 500 flies of each sex; more than 1,000 flies of each sex were measured at the control temperature, 28°. There is a pronounced difference between the males and females in bristle numbers, the former having a lower mean for all bristles at all temperatures. The sexes are therefore treated separately. These results are shown graphically in figures 2 to 6. The curves drawn through the points are merely to aid the eye in following the slopes; they are of no theoretical significance.

The effect of temperature is not the same on all the bristles of the scute-1 flies. With increasing temperature there is an increase in mean numbers of the aor, oc, anp, apa, iv, and por; a decrease in the psc, and adc; the asc, and pv reach maxima at 20.0° and 24.5° respectively; the mor and the sum of the aor and mor reach minima at 28.0°. The coxal-1 bristles, on the coxae of the first pair of legs, were never found at any temperature studied. The humeral and sternopleural bristles seemed to be affected by temperature but they were not counted extensively.

PAYNE (1920) reported that in his high selected "reduced" (undoubtedly from his description, some scute allele) there was a decrease in scutellar bristles with increasing temperature. If the present results on the scutellar bristles are extrapolated to the temperature at which PAYNE observed an

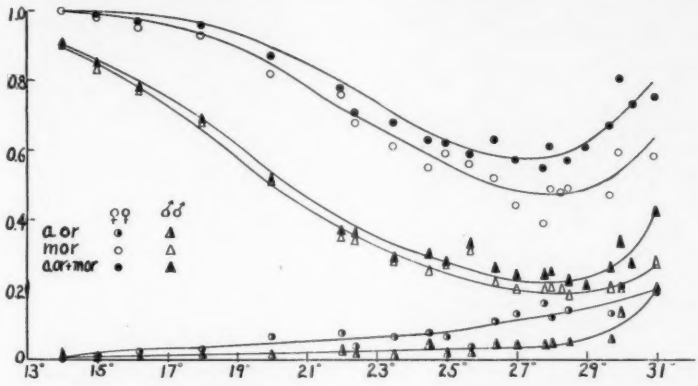

FIGURE 2.—Effect of temperature on mean bristle number of scute-1. Ordinates, mean bristle number per half fly; abscissae, temperature in °C.

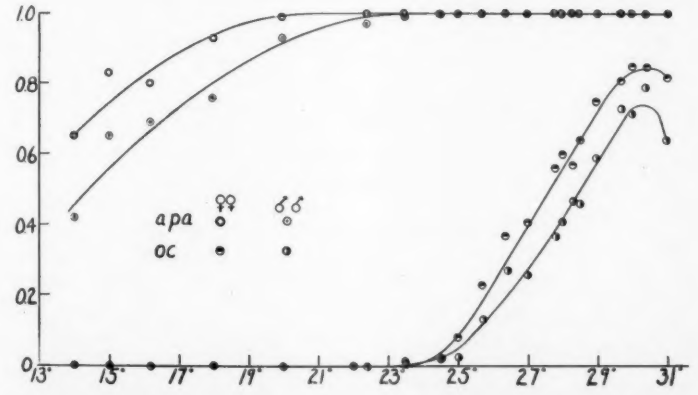

FIGURE 3.—Effect of temperature on mean bristle number of scute-1. Ordinates, mean bristle number per half fly; abscissae, temperature in °C.

increase in scutellar bristles (10°–13°), the sum of the asc and pac is greater than their sum at the higher temperature (23°–26°) studied by PAYNE. METZ (1920) in analyzing the effect of temperature on bent in *D. virilis* and *D. melanogaster* found that at low temperatures there was a decrease in the scutellar bristles of the former species but an increase in the latter.

PLUNKETT (1926) reported that in Dichaete there was a decrease in asc at high temperatures.

The effect on the adc bristles is the same as reported by PLUNKETT (1926), a decrease with increasing temperature. For these few bristles it appears

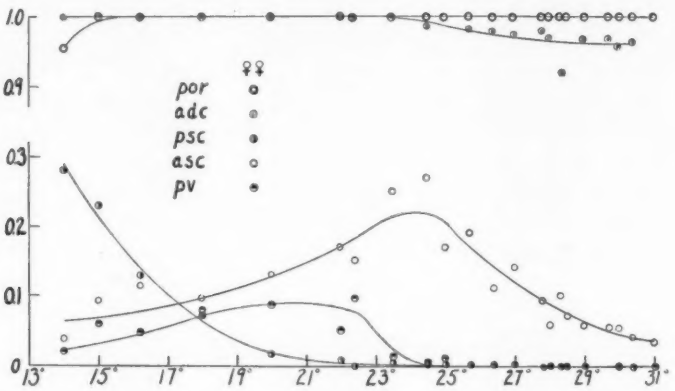

FIGURE 4.—Effect of temperature on mean bristle number of scute-1. Ordinates, mean bristle number per half fly; abscissae, temperature in °C.

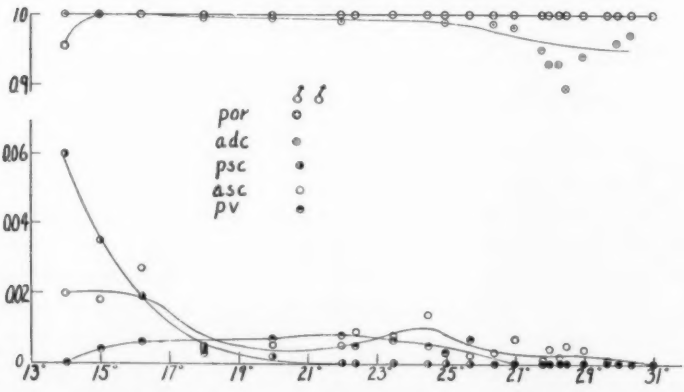

FIGURE 5.—Effect of temperature on mean bristle number of scute-1. Ordinates, mean bristle number per half fly; abscissae, temperature in °C.

that each particular bristle is affected in the same direction by temperature, even in different mutants. This is not considered necessarily significant since the data on other mutants are too few to make an extensive comparison.

The great changes in slope found, for example, in the oc (figure 3) and anp (figure 6) are not uncommon in temperature studies on Drosophila. HARNLY (1929) and STANLEY (1930) in their studies on wing length of vestigial flies reported that between 29° and 32° there was a great increase in wing length; at lower temperatures between 15° and 29° the increase in size of the wing is very slight. HERSH (1925), studying the effects of temperature on Bar heterozygotes, reported a marked change in facet number between 27° and 28°.

The effect of temperature on the bristles of scute-1 as shown by the data here presented is evidently quite complex, each bristle behaving differently from the others. No generalization as to this effect seems possi-

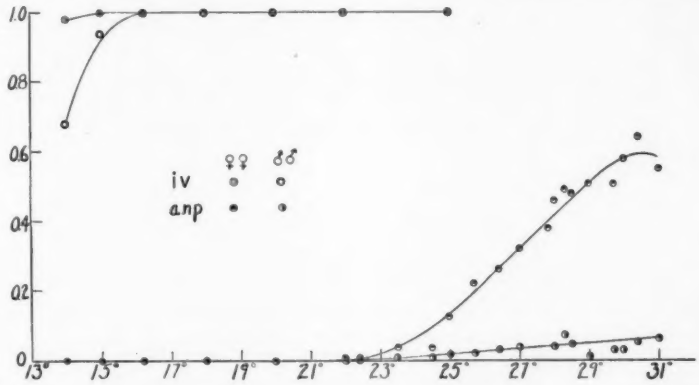

FIGURE 6.—Effect of temperature on mean bristle number of scute-1. Ordinates, mean bristle number per half fly; abscissae, temperature in °C.

ble except that every bristle which is at all variable is affected by temperature, at least in some part of the temperature range.

It is also obvious from these data that certain bristles (mor, oc, por, iv, apa, adc) are apparently not affected at all by the scute-1 gene (that is, do not differ phenotypically from the wild-type) at some temperatures, but are markedly affected at other temperatures.

DISCUSSION

The sub-gene hypothesis

These results may be used as a test of certain theories which have been advanced to explain the effect of scute on the bristles of *Drosophila melanogaster*.

In a series of papers DUBININ (1929, 1932), SEREBROVSKY (1930), LEWIT (1930), AGOL (1930, 1931) and their colleagues have described sixteen

other acute alleles, each differing from the others by removing certain definite bristles and not affecting other bristles. As a result of their careful studies they have elaborated an interpretation of the nature of the scute locus. According to their hypothesis the normal allele of the scute gene, the "basigene," is not a single unit but it is divided into a number of elementary units called "sub-genes" or centers, arranged in a linear order in the scute locus. Each center is concerned with the development of a certain one or few bristles of the fly. Each scute allele, according to their interpretation, represents a mutation in some one or more of these centers, and only one kind of mutation is possible in any one center.

The bristles of the fly have been arranged in a linear series which corresponds to the postulated linear order of the sub-genes. STURTEVANT and SCHULTZ (1931), in presenting evidence against the sub-gene hypothesis,

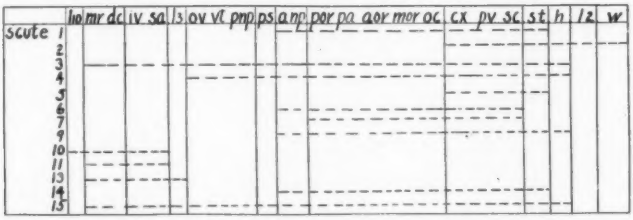

FIGURE 7.—(After DUBININ and FRIESEN 1931) The scute step-alleles 1_{10}, 1_3, 1_2=lethals associated with scutes-10, -3, and -2 respectively.
mr=microchaetes on the thorax
vt=ventral bristles between the first pairs of legs
cx=coxal bristles
w=crumpled wings
The other abbreviations are the same as those used previously (figure 1).

have constructed a slightly different seriation based on their own data. Using this series as a base line they have drawn a curve for each allele, plotting as ordinates the mean bristle number for each bristle. That most of the curves are smooth and unimodal is taken as evidence that the seriation is of some significance.

Figure 7 (DUBININ and FRIESEN 1932) illustrates the seriation as postulated by the proponents of the sub-gene hypothesis. As can be seen from this chart, scute-1 is interpreted as being a mutation involving four centers therefore affecting the bristles determined by these four.

In figure 8 the means of each bristle from my data have been plotted against this seriation of the bristles. The limits of the centers are shown by the black solid lines. At 22.0° which is probably approximately the temperature at which the Russian workers raised their flies, the

bristles affected in my experiments are the same as found by them with one exception, the por, which is affected at lower temperatures. At 14° the iv center affected by scutes-3, -10, -11, and -15 is affected by scute-1, and the mor, which according to their hypothesis is affected by scute-1, is not affected in the females. At higher temperatures there is a decrease in M adc and therefore the postulated dc center which they find affected by scutes-3, -10, -11, -13, and -15, is now being affected by scute-1; whereas the apa in one of the postulated affected centers is unaffected in both males and females. These facts show that bristles outside the centers postulated for scute-1 may be affected by merely changing the temperature, and that bristles in the mutated centers postulated for scute-1 may be unaffected at some tem-

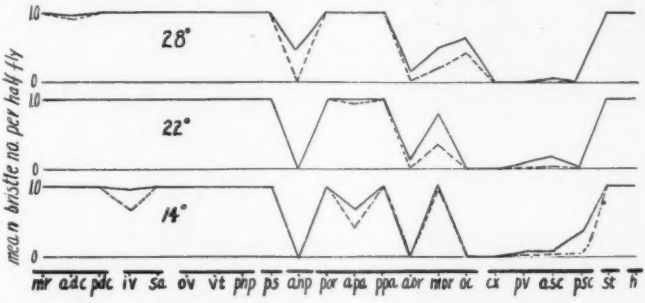

FIGURE 8.—Mean bristle numbers *vs.* seriation of DUBININ et al.
— = ♀ ♀ · · · = ♂ ♂ when differing from ♀ ♀.

peratures. A few preliminary experiments on other scute alleles show similar exceptions. These results seem to remove the essential basis of the sub-gene hypothesis.

STURTEVANT and SCHULTZ (1931), studying various scute alleles in the presence of duplicated fragments of the X chromosome, found that in these instances the effect of scute on the bristles is a function of the genetic system as a whole, including modifiers in other loci as well as the scute locus itself. Evidence bearing on the validity of their conclusion was obtained during the course of selection of the scute stock used in my experiments. It was found that it was possible to select for certain bristles without affecting others. In fact I found that after the stock was made isogenic for modifiers affecting the oc and mor bristles, the anp still showed significant positive left-right and parent-offspring correlations. The anp bristle was then selected for and the stock was made isogenic for modifiers affecting it without changing the means of the other bristles. There was no selection for the iv, adc, and apa and table 1 shows that the stock is

not uniform for factors modifying these characters. The conclusion reached by STURTEVANT and SCHULTZ, that the effect of scute on bristles is a function of the genetic system as a whole rather than merely the single scute locus, is supported by these results.

Experiments of another nature also support the validity of this conclusion. PLUNKETT (1926) found that two genes neither of which separately removes certain bristles may do so when combined. It appears therefore that the action of these genes is less specific than might at first appear. It seems as though each gene has a tendency to remove more bristles than it ordinarily does but the effect is not strong enough to remove the bristles

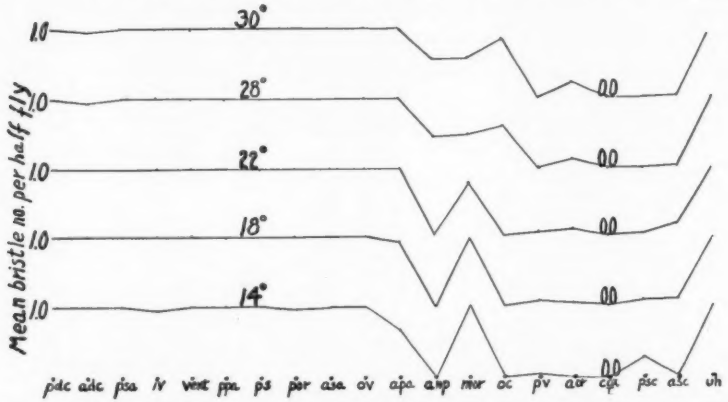

FIGURE 9.—Mean bristle numbers of scute-1 ♀ ♀ *vs.* seriation of STURTEVANT and SCHULTZ (1931).

unless intensified by the action of another bristle-reducing gene. STURTEVANT and SCHULTZ (1931), using this method of intensifying the action of one gene by another, showed that scute-1 in combination with Hairless removed many more bristles than either would when acting separately. One of the bristles removed was the iv which is not affected by scute-1 at ordinary temperatures but is affected at very low temperatures (figure 6).

These results of STURTEVANT and SCHULTZ and my own observations show quite clearly that the sub-gene hypothesis is inadequate in that it does not meet these experimental tests. Despite their conclusion with respect to the sub-gene hypothesis, STURTEVANT and SCHULTZ still thought that their seriation of the bristles was of some significance. They based their belief in the fact that they obtained smooth unimodal curves when the bristle numbers were plotted against this seriation. In figure 9 the means for each particular bristle have been plotted in this way (using the seriation of STURTEVANT and SCHULTZ) at several temperatures. It

can be seen from this figure that it would be necessary to change the seriation for almost every change in temperature in order to obtain smooth unimodal curves in all cases. These facts seem to raise a serious doubt that the seriation is of any significance.

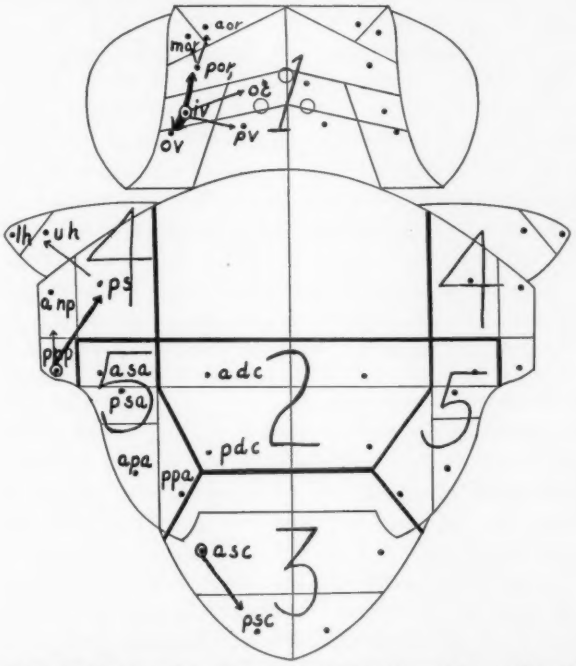

FIGURE 10.—(Modified from GOLDSCHMIDT 1931) The fields are delineated by the heavy lines. The arrows indicate the direction diffusion of the bristle-determining substance from the centers in these fields; the diffusion occurring first along the path of the heavier arrows before it takes place along the path of the lighter arrows.

The diffusion hypothesis

GOLDSCHMIDT (1931) has advanced an hypothesis to explain the behaviour of the scute series of alleles. The essential points of his theory are as follows:

1. There are present on the fly five fields within each of which a bristle-determining substance diffuses in definite patterns from centers in these fields (figure 10).

2. The period in development when the diffusion of this bristle-determining substance can be initiated differs for the different centers of the fly.

3. The diffusion from a center is initiated by the presence of a minimal

amount of a second substance. This minimal amount must be present at a time in development which falls within the part of the period during which the diffusion from that center can be initiated "Die Zeitspanne der Öffnung der Austrittstellen des Stroms" (GOLDSCHMIDT 1931, p. 530). If it is present before the diffusion can take place it will not remain to initiate the diffusion process at a later period.

4. The rate of formation of this initiating substance is determined by the scute allele or alleles present in the fly.

Figure 10 shows the location of these fields and centers as postulated by GOLDSCHMIDT. Following the suggestion by PLUNKETT (1926), STURTEVANT and SCHULTZ (1931) had also considered a diffusion hypothesis for scute. They thought that a single center in the median line near the pdc was sufficient to explain the pattern of bristle determination on the whole fly.

In describing the diffusion of bristle-forming substance on the head of the fly, GOLDSCHMIDT (1931, p. 513) states, "In der Kopfregion liegt diese Austrittstelle offenseitlich jederseits in der Region der vordern Verticalborsten," (the iv in figure 10). "Von hier dringt der Strom zuerst nach hintern zu den hintern Verticalborsten (ov in figure 10) und dann zu den dritten Orbitalborsten" (por in figure 10) . . . "Vom 3 Orbitalfeld aus geht der Strom nach vorn, und vom Verticalfeld nach der Mittelinie . . . ." This is shown by means of the arrows in figure 10 as explained by the legend. The bristle-determining substance is therefore postulated to be at its greatest concentration around the iv, from which center the other regions on the head receive their bristle-determining substance. As a logical deduction from this postulate it follows that if the M iv is low, the mean numbers of the other bristles on the head must be correspondingly reduced. At 14°, however, in the females the M iv = 0.96 and the M mor = 1, in the males the M iv = 0.68, and the M mor = 0.90. This seems to be a direct contradiction of the result to be expected if the iv were the center with the greatest concentration of bristle-determining substance from which the mor received this substance by diffusion. Another contradiction is found at low temperatures. The M psc being greater than the M asc, the asc being the location of the postulated center of the scutellar field. DUBININ and FRIESEN (1932) point out similar contradictions with respect to other scute alleles. From the fact that he does not locate the centers in the fields 2 and 5 (figure 10), it appears that GOLDSCHMIDT himself thought that this part of his theory was weak.

The data on scute-1 in the present paper show in another way the inapplicability of any diffusion hypothesis. If this process affected the distribution of bristle-forming substances, it should be expected that the bristles in a field would show some degree of association with each other.

Table 2 showed that there was no association between any of the bristles in scute-1 (except for the special case of the aor-mor which has been discussed previously).

These results indicate that in scute-1 the distribution of bristles on the fly is not governed by the diffusion of bristle-forming substances from a few centers, but that each bristle varies independently of the others. Each bristle has its own separate center, if we chose to express the facts in such terms, where processes go on that determine whether the bristle shall be present or absent. (This does not necessarily imply that the primary action of the scute gene itself is strictly localized for each bristle.)

This conclusion, which rejects the entire concept of pattern as applied to scute, may appear somewhat radical to those who have observed these flies. It is not difficult to see how the concept arose that in scute there is a certain pattern of bristle distribution. If the mean bristle numbers and not the individual flies are considered, the impression one obtains is that certain bristles are affected more than others, in a definite pattern. When the individuals are considered separately, however, it is seen that there is no consistent pattern. The distribution of bristles on one fly may differ in any way from that of another fly. This has been shown by the fact that there is no association between the bristles. When the flies raised at different temperatures are compared with each other the concept of a general pattern in scute becomes even more inapplicable. Flies raised at 14° show variations in some bristles which are constant at higher temperatures, and *vice versa*. These flies appear as different from each other as do two different scute alleles raised at the same temperature.

These data show that the concept of pattern of distribution in scute is based on nothing more than differences in the mean numbers, in a group of flies, of different bristles and does not correspond to any developmental processes in the individual fly.

These results are in striking contrast to those found by PLUNKETT (1926) in Dichaete flies. In Dichaete it was found that a diffusion hypothesis could be included as part of the developmental theory explaining the phenotypic effect of this mutant gene as compared with its wild-type allele. A definite pattern of bristle distribution was found with association coefficients of 1 between bristles very close to one another, adc–pdc, and lh–uh. The associations between pairs of bristles further away from one another than the examples cited were less than one, but still significantly greater than 0. This is quite logically explained by PLUNKETT (p. 234) as due to "diffusion from a center but with considerable random local variation in its course. Such irregularities in diffusion are by no means inconceivable when it is realized that we are dealing with a substance of probably high molecular weight, colloidal dimensions, and present in a

very small quantity, diffusing through the physically heterogeneous medium of a highly differentiated egg or a multicellular embryo."

Although quite unessential to this theory, GOLDSCHMIDT thought that the period during which the diffusion can be initiated is the same as the period during which temperature is effective in changing the phenotypic expression of the gene. The fact that in my experiments, as will be reported in a later communication, it is found that the effects of temperature on all bristles (with certain few exceptions) occur during the same time does not detract from the theory. It may very well be that temperature is effective in changing the bristle number by affecting some processes earlier in development, which process occurs at the same time for all bristles.

The present data on scute-1 have no bearing, one way or another, on the remaining postulates of GOLDSCHMIDT's theory. These have been discussed by DUBININ and FRIESEN (1932), whose results seem to cast serious doubts on the validity of this entire theory.

The general "rate" theory of gene action

GOLDSCHMIDT (1927), from his work on intersexuality in Lepidoptera, and PLUNKETT (1926), from his analysis of Dichaete in *Drosophila melanogaster*, have elaborated the general theory that genes act by affecting the rates of developmental processes in the organism. The effects of temperature on the bristle number of the wild-type are very slight and found only near the extremes of the temperature range in which the flies will develop to maturity at all (these were not extensively studied, and are therefore not included in the data). The bristles of the scute fly, however, are markedly affected by temperature, every bristle which is at all variable being affected in at least some part of the temperature range.

The difference in effects of temperature, and also of other environmental factors, on the bristles of scute and those of the wild-type, is ascribed, on the basis of the general theory, to the differences in the effects of these environmental factors on the rates of developmental processes leading to bristle formation in scute and the wild-type. Thus in scute flies, either the rates of the processes or the periods during which these processes go on, or both, must differ at different temperatures to produce different bristle numbers. In view of the nature of the experimental material there is no means of determining the rate independently of the duration, whereas the duration of the processes differentially affected by temperature may be independently determined. The duration of these processes have been determined for scute-1 and a discussion of the bearing of these results on the general theory of GOLDSCHMIDT and PLUNKETT must await the presentation of these data in a subsequent paper.

SUMMARY

1. A scute-1 stock inbred and selected for 22 generations was made isogenic for genetic modifiers of the bristles selected but not for the other bristles.

2. With but one exception no association was found among the bristles on the same side of the fly.

3. The exception, aor-mor was found to have an association coefficient of -1, and the possibility is discussed that the bristle recorded as aor was the mor shifted in position.

4. The bristle numbers of the males were lower than those of the females under all conditions.

5. With an increase in temperature the mean numbers of some bristles increase, some decrease, some reach maxima, and others minima.

6. These results on scute-1 seem to remove the essential basis of the sub-gene hypothesis and to cast grave doubts on the significance of any seriation of bristles.

7. The concept of a pattern of bristle distribution, whether determined by diffusion or by any other mechanism, seems to be inapplicable to scute-1.

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PHENOGENETIC STUDIES ON SCUTE-1 OF DROSOPHILA MELANOGASTER.

II. THE TEMPERATURE-EFFECTIVE PERIOD

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Received March 16, 1934

INTRODUCTION

The effects of temperature on the mean bristle numbers of scute-1 are reported in the preceding article. The present paper is concerned with a study of the temperature-effective periods of these bristles to determine:

(a) at what period during development does temperature affect bristles,

(b) is this time the same for all the bristles,

(c) is the time the same at all temperatures relative to the time of development as a whole?

EXPERIMENTAL

Material and methods

The culture methods and the tests for the homogeneity of the stock used in these experiments were reported previously. It should be added that where rapid changes in temperature were desired as in shifting cultures from one temperature to another, water baths were used to shorten the transition periods.

Effect of temperature on time of development

It will be shown in the next section that the effect of temperature on the bristle number in scute-1 is confined to a definite period during larval life. To determine whether this "temperature-effective period" is the same portion of the whole egg-larval period (that is, the period from egg-laying to pupation) at all temperatures, it is necessary to have accurate data on the duration of these stages at each temperature.

In most experiments the eggs were laid at the temperature at which they were to develop. At very high and very low temperatures the eggs were laid at 25° during a very short period. At high temperatures, 30° and 31°, the error introduced is very slight since the times of development at 25° and at these high temperatures are quite similar. At low temperatures, 14° and 18°, the error may be much greater, depending upon the duration of the egg-laying period. A very simple factor was employed at these temperatures to correct for the egg-laying period. Let t_2 be the time of development at the egg-laying temperature and t_1 the time of development at the lower temperature. If d is the duration of the egg-laying period

the apparent time of development t_1', experimentally obtained at the lower temperature, must be increased by the quantity

$$d\frac{t_1-t_2}{t_2}$$

or

$$t_1=\frac{(t_1'-d)t_2}{t_2-d}.$$

If d is very small $t_1=t_1'$ approximately. The values in table 1 have been corrected in this way where necessary.

TABLE 1

Effect of temperature on duration of egg-larval period.

TEMPERATURE	♂♂	TIME (HOURS)	♀♀
15.0°	369.1±2.75		377.7±2.29
16.2°	291.3±1.42		300.6±2.18
18.0°	229.1±1.02		236.9±0.46
20.0°	178.6±0.46		185.4±0.42
22.4°	129.4±0.39		136.8±0.29
25.0°	101.3±1.22		109.7±0.85
27.0°	94.8±0.40		98.6±0.42
27.6°	90.0±0.43		94.0±0.31
28.0°	89.6±0.13		94.1±0.15
28.6°	89.3±0.14		92.7±0.23
29.0°		92.7*	
29.7°	95.3±0.20		98.5±0.26 (σ=3.18
30.0°	96.2±0.14		99.5±0.12 hrs.)
31.0°		112.0*	

* The sexes were not determined separately in these experiments.

From the data (table 1) it can be seen that at all temperatures the duration of the egg-larval period for the males is shorter than for the females. A similar result has been found by Mr. LOUIS POWSNER in this laboratory (unpublished) in a wild stock unrelated to the scute-1 used in these experiments. BONNIER (1926), however, reported that in his stock at 30° the duration of the egg-larval period for the females was less than for the males, while at 25° there was no significant difference between the two sexes.

Temperature-effective period

In order to determine during which period of development, larval or pupal, the temperature was affecting the bristle number, two preliminary experiments were performed. In the one the flies were allowed to develop up to pupation at 20° and then transferred to 28° to complete their development; in the other the reciprocal transfer was made. The results are presented in tables 2 and 3.

TABLE 2

♂♂

Effects of temperature. Egg-larval and pupal periods, respectively, at temperatures shown.

BRISTLE	EGG-LARVAL PERIOD AT	PUPAL PERIOD AT	MEAN BRISTLE NO.	DIFFERENCE	S.E. OF DIFFERENCE
aor	20.0	28.0	0.03	+0.015	0.005
	20.0	20.0	0.015		
aor	28.0	20.0	0.029	+0.013	0.015
	28.0	28.0	0.042		
mor	20.0	28.0	0.52	+0.01	0.022
	20.0	20.0	0.51		
mor	28.0	20.0	0.27	+0.05	0.030
	28.0	28.0	0.21		
oc	20.0	28.0	0.00	0.00	0.00
	20.0	20.0	0.00		
oc	28.0	20.0	0.46	−0.05	0.038
	28.0	28.0	0.41		
anp	20.0	28.0	0.00	0.00	0.00
	20.0	20.0	0.00		
anp	28.0	20.0	0.058	−0.021	0.014
	28.0	28.0	0.037		
asc	20.0	20.0	0.013	−0.08	0.0031
	20.0	20.0	0.005		
asc	28.0	20.0	0.006	+0.002	0.0049
	28.0	28.0	0.004		
psc	20.0	28.0	0.004	−0.002	0.0019
	20.0	20.0	0.002		
psc	28.0	20.0	0.00	0.00	0.00
	28.0	28.0	0.00		
pv	20.0	28.0	0.002	+0.005	0.0019
	20.0	20.0	0.007		
pv	28.0	20.0	0.00	−0.0003	0.0003
	28.0	28.0	0.0003		
apa	20.0	28.0	0.89	−0.04	0.011
	20.0	20.0	0.93		
apa	28.0	20.0	1.00	0.00	0.00
	28.0	28.0	1.00		

TABLE 3

♀♀

Effects of temperature. Egg-larval and pupal periods, respectively, at temperatures shown.

BRISTLE	EGG-LARVAL PERIOD AT	PUPAL PERIOD AT	MEAN BRISTLE NO.	DIFFERENCE	S.E. OF DIFFERENCE
aor	20.0	28.0	0.062	−0.004	0.012
	20.0	20.0	0.066		
aor	28.0	20.0	0.091	−0.011	0.023
	28.0	28.0	0.012		
mor	20.0	28.0	0.86	−0.04	0.019
	20.0	20.0	0.82		
mor	28.0	20.0	0.47	−0.02	0.035
	28.0	28.0	0.49		
oc	20.0	28.0	0.00	0.00	0.00
	20.0	20.0	0.00		
oc	28.0	20.0	0.60	0.00	0.034
	28.0	28.0	0.60		
anp	20.0	28.0	0.00	0.00	0.00
	20.0	20.0	0.00		
anp	28.0	20.0	0.40	+0.06	0.035
	28.0	28.0	0.46		
asc	20.0	28.0	0.11	+0.02	0.016
	20.0	20.0	0.13		
asc	28.0	20.0	0.096	+0.04	0.017
	28.0	28.0	0.056		
psc	20.0	28.0	0.021	−0.004	0.0064
	20.0	20.0	0.017		
psc	28.0	20.0	0.00	0.00	0.00
	28.0	28.0	0.00		
pv	20.0	28.0	0.034	+0.052	0.014
	20.0	20.0	0.086		
pv	28.0	20.0	0.00	−0.0007	0.0007
	28.0	28.0	0.0007		
apa	20.0	28.0	0.991	−0.001	0.0049
	20.0	20.0	0.990		
apa	28.0	20.0	1.00	0.00	0.00
	28.0	28.0	1.00		

The differences between the mean bristle numbers of flies transferred from one temperature to the other at the time of pupation and of flies allowed to develop entirely at the one temperature are shown. The positive sign means that the result of the transfer is in the direction that would be expected if the second temperature had any effect. The differences in most cases are small and not significant when compared with their standard errors. These results indicate, therefore, that the temperature-effective period occurs during the egg-larval stage of development. Data on the iv and por have not been extensively collected, but transfers made after flies, raised at low enough temperatures to show these effects, have pupated, indicate that the temperature-effective period for these characters also occurs in the larval period.

The limits of the temperature-effective period during egg-larval life were determined by transferring flies at intervals during their larval period from one temperature to another. In order to ascertain whether the temperature-effective period occupied the same portion of the egg-larval life at all temperatures, a number of different initial and final temperatures were used. The figures (1–4) show the results of these experiments. To make curves comparable with one another they were all plotted on the same base line, fraction of egg-larval life spent at the first temperature T_1 before being transferred to the second temperature T_2 for the completion of their development. The curves drawn through the points are merely to aid the eye in following the slope. They are of no theoretical significance.

Examination of the curves, figures 1–4, shows that in general the temperature-effective period for these bristles occurs, with some slight variation, during the same period, 75 percent–98 percent of egg-larval life, at all temperatures.

PAYNE (1920) reported that in a rough experiment performed by one of his students, ALBERT FROEMMING, temperature was without effect on the scutellar bristles of "reduced" in the pupal period. METZ (1926) found a similar result on scutellar bristles affected by bent both in *D. virilis* and *D. melanogaster*. PLUNKETT (1926), however, reported that the temperature-effective period for the dorso-central bristles studied in Dichaete continued for some time into the pupal period.

Figure 2, showing the effects on the anp of transfers from lower to higher temperatures, discloses a very unexpected phenomenon: transfers made during the period comprising 50 percent–75 percent (within the limits of experimental error) of egg-larval life, produce flies with mean anp numbers higher than the means of flies raised at either temperature throughout their larval life. This is shown most strikingly in the males (figure 2) since at all temperatures the M anp is very low (CHILD in press).

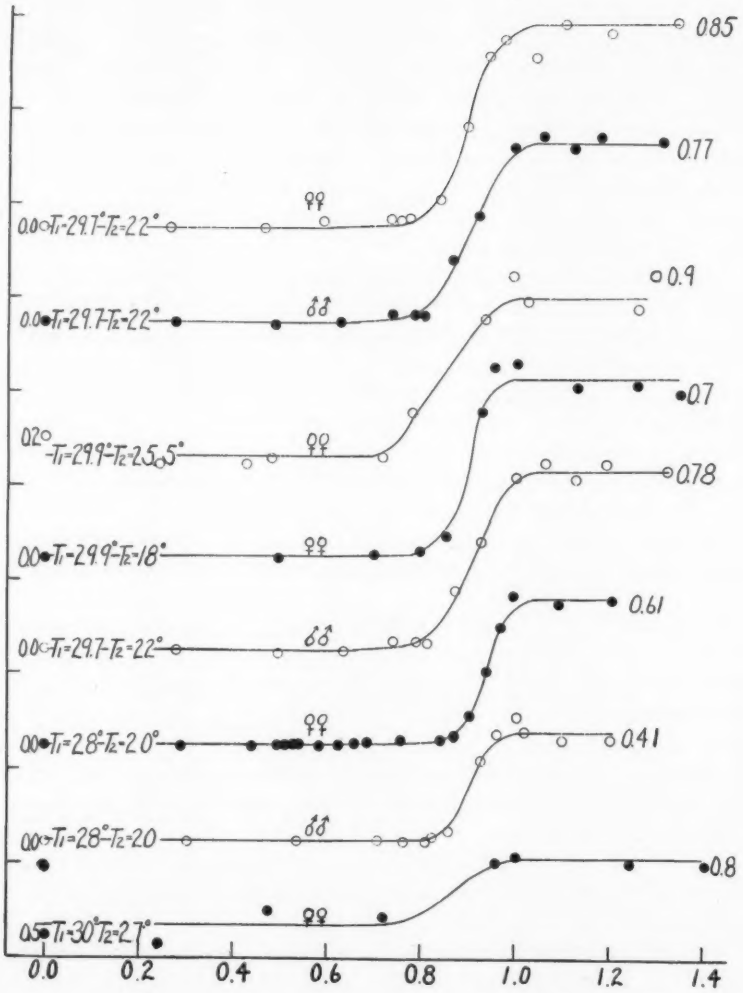
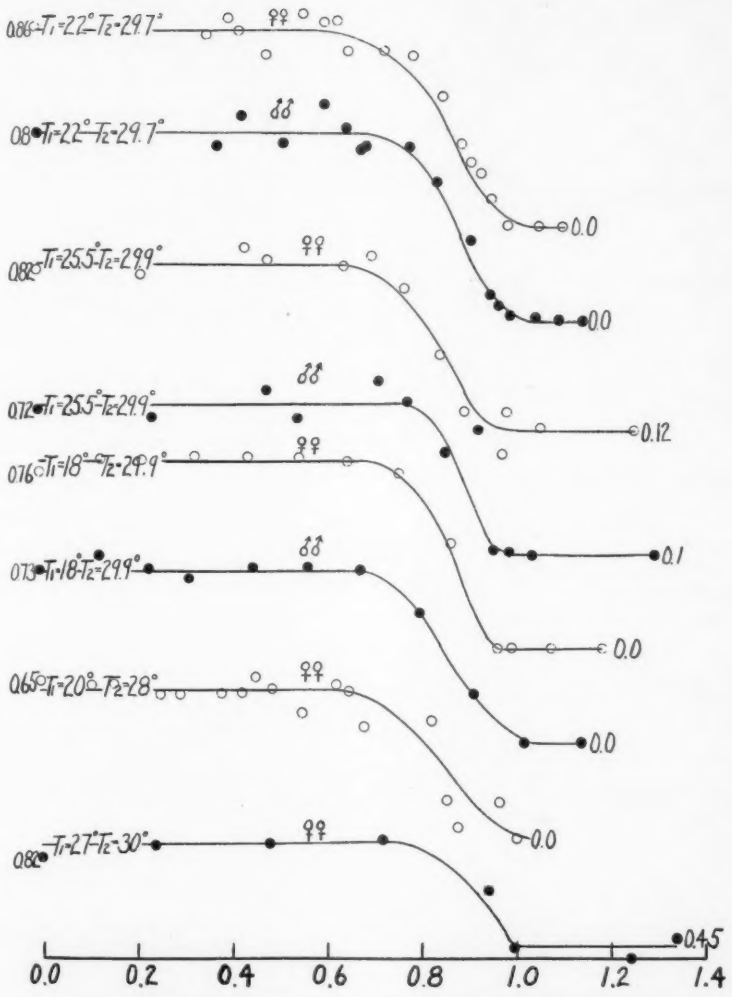


FIGURE 1.—Temperature-effective period of the ocellar bristle ordinates M oc;



abscissae fraction of egg-larval life spent at T_1 before being transferred to T_2.

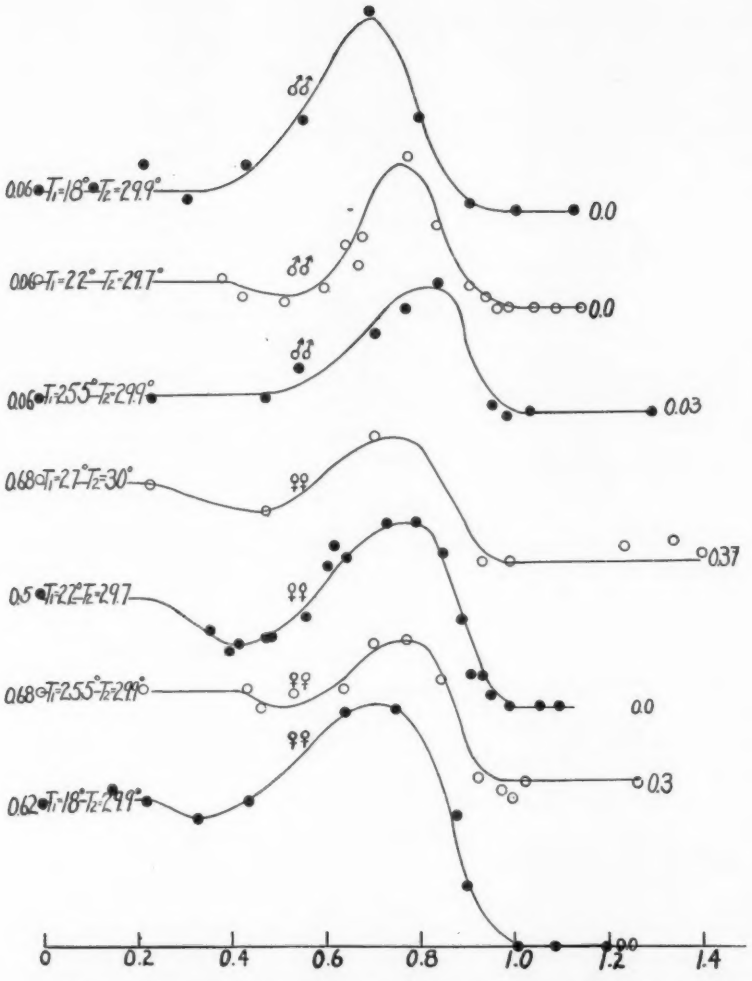
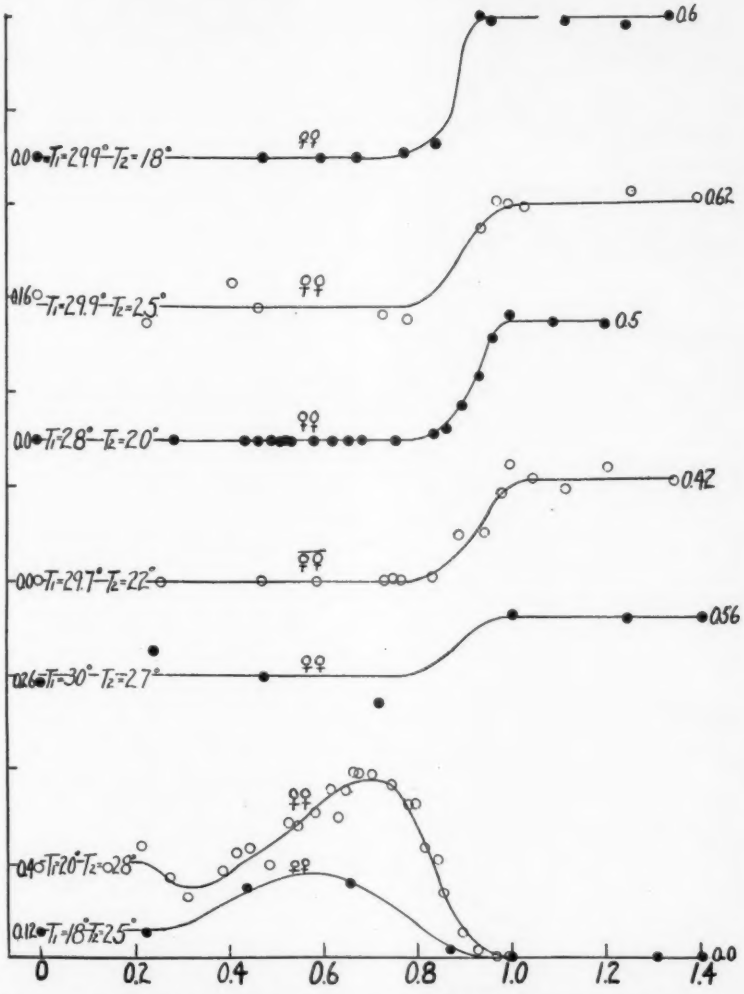


FIGURE 2.—Temperature-effective period of the anterior notopleural bristle ordinates



M anp; abscissae fraction of egg-larval life spent at T_1 before being transferred to T_2.

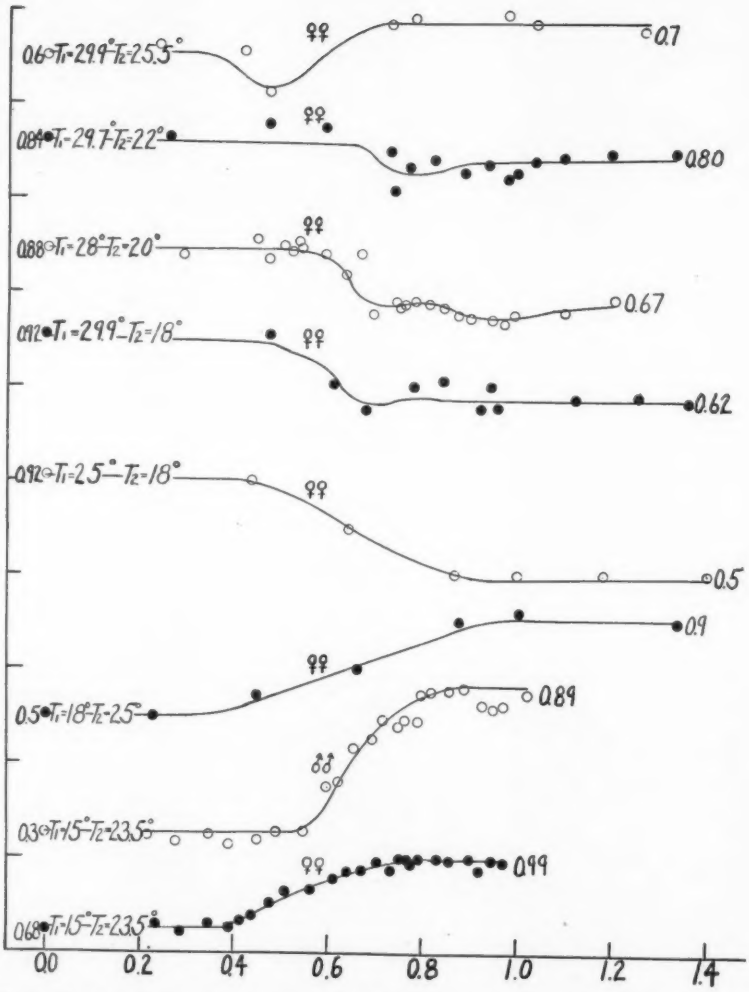
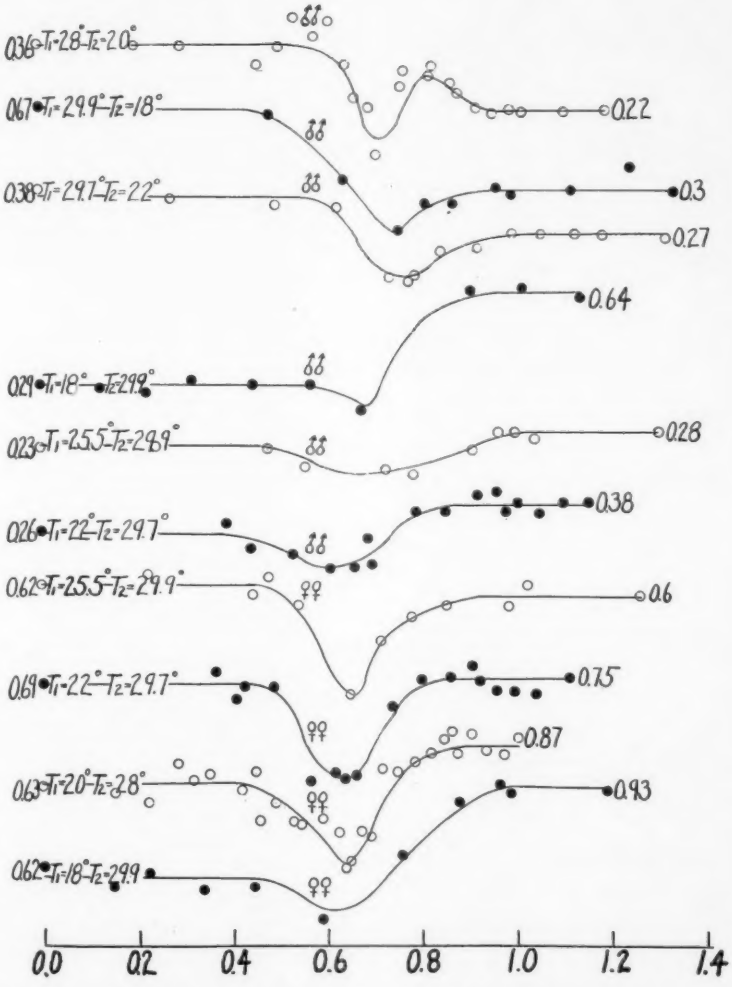


FIGURE 3.—Temperature-effective period of the anterior and middle orbital bristles ordinates



M aor+mor; abscissae fraction of egg-larval life spent at T_1 before being transferred to T_2.

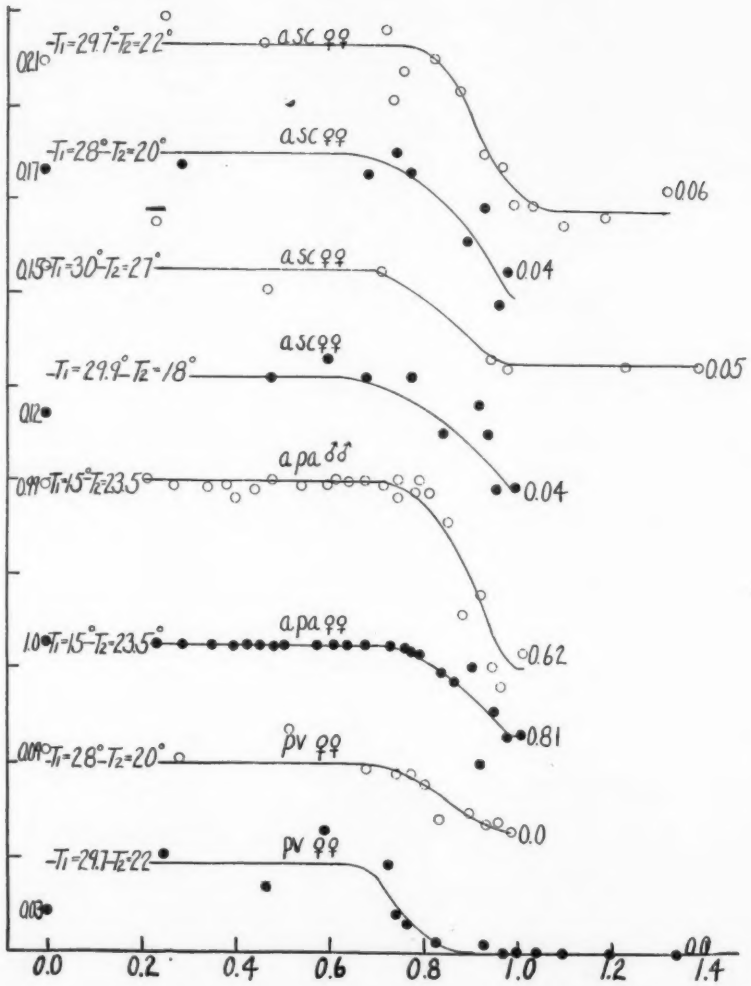
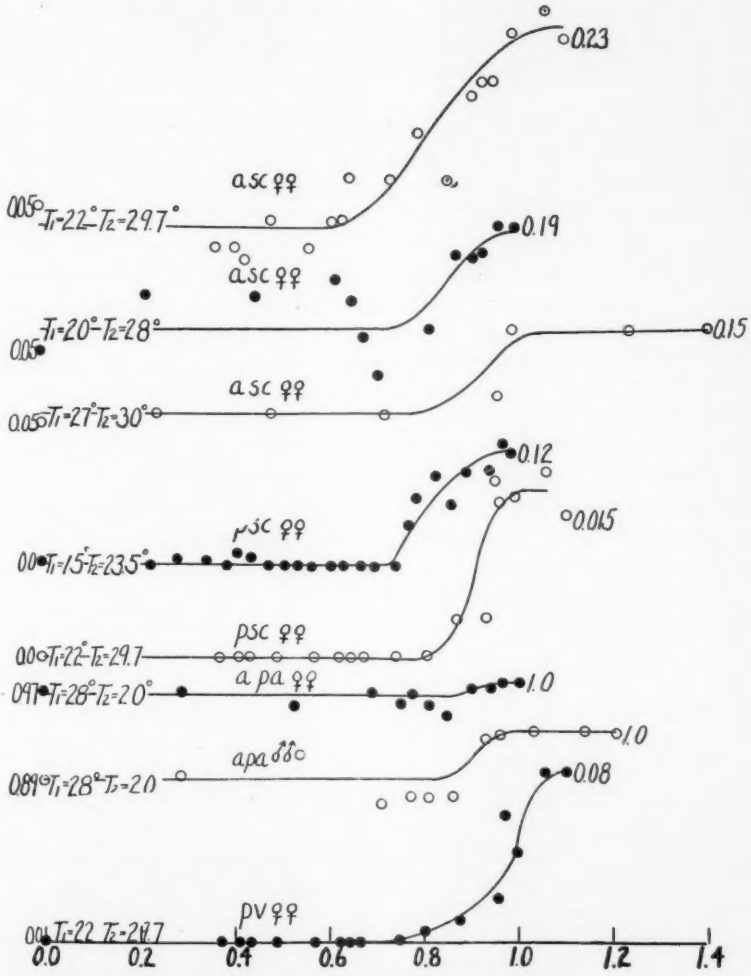


FIGURE 4.—Temperature-effective period ordinates mean bristle number; ab-



scissae fraction of egg-larval life spent at T_1 before being transferred to T_2.

After this rise there is a fall in bristle number, the period of which is the same as the temperature-effective period for the bristles mentioned previously. In some of the experiments there appears to be a short period prior to the rise during which there is a fall in bristle number. This, however, is not always obtained and may not be of any significance. None of these peculiarities appear when flies are transferred from high to low temperatures.

The results for the aor+mor bristles are even more complex than for the anp. Two temperature ranges have been chosen with reference to the minimum obtained at 28° when flies are raised at constant temperature for their total period of development: (a) a range below, and (b) a range including 28°. When the initial and final temperatures are on one side of the minimum (a), the results obtained (figure 3) are like those described above for most bristles. The temperature-effective period, however, is not the same as that for the other bristles since it occupies the period comprising 55 percent–80 percent of egg-larval life. When the temperature range includes 28°, T_1 and T_2 being on opposite sides of the minimum, another kind of curve is obtained (figure 3). Flies transferred between 55 percent–70 percent of egg-larval life (within the limits of experimental error), show a decrease in M aor+mor, reaching a minimum value which is lower than the values in flies raised at either temperature throughout their larval life. The rest of the temperature-effective period covers the 70 percent–85 percent range of egg-larval life, the mean numbers rising to their values at T_1. These peculiarities occur equally when transfers are made from lower to higher or from higher to lower temperatures.

From these data on temperature-effective periods, two general results stand out. (1) The temperature-effective period for any particular bristle occupies the same relative portion of the whole egg-larval period at all temperatures, that is, when the times of beginning and ending of the temperature-effective periods are expressed as fractions of the whole egg-larval period (as in figures 1–4) these points coincide at all temperatures. (2) With the exceptions of the peculiar effects on the anp and orbital bristles, the temperature-effective period is the same for all bristles.

These conclusions can, of course, be regarded as valid only within the limits of accuracy of the experimental determinations of the temperature-effective period.

This method of determining the temperature-effective period gives the limits of the period for the whole population being studied, that is, the period from the time at which the first flies start to be affected by temperature to the time at which this effect has been completed in all the flies. The temperature-effective period as measured (P), is, therefore, the sum of two quantities: the mean duration of the temperature-effective period

in the individual flies (D), and the variation (V) from fly to fly in the time at which the mid-points of D are reached. There are three possible interpretations of these data as shown schematically in figure 5.

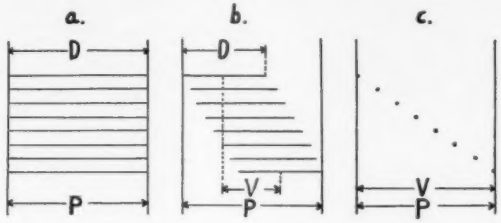


FIGURE 5.—Schematic representation of the three possible interpretations of the temperature-effective period as measured.

Figure 5 (a) shows the interpretation of the temperature-effective period which is implied in the treatment employed by the investigators working on Bar, O. DRIVER (1931), E. DRIVER (1926, 1931), KRAFKA (1924), LUCE (1931), and by PLUNKETT on Dichaete; that is, the period for each fly, indicated by the horizontal lines, starts and ends at the same time

$$\therefore\ D=P,\ V=O$$

in figure 5b

$$P>D>O,\ P>V>O,\ P=D+V$$

in figure 5c

$$V=P,\ D=O$$

that is, D is merely a point in time, or in other words the effect of temperature in any individual is practically instantaneous.

Since the temperature-effective period may be interpreted in any of these three ways, the problem is to determine which interpretation best fits the experimental results.

A preliminary experimental attack was made on this problem as follows: at 28°, for an egg-laying period of one hour, in a large population of flies, the spread in time of pupation covers a period of 20 hours (this does not include the larvae which pupate long after the rest of the larvae have pupated; these larvae seldom complete their development and when they do, are usually abnormal in appearance) and gives an approximately normal distribution (figure 6). At any particular moment during this period of pupation the developing individuals may be divided into four easily distinguishable classes: (1) "old" pupae (brown), (2) "young" pupae (white), (3) motile larvae with everted anterior spiracles, (4) larvae with-

out everted spiracles. An experiment was performed in which flies, during this period, were separated into these four groups and transferred at the same time to 20°. The results of this experiment indicate that the first three classes had passed through their temperature-effective periods, whereas all the larvae of group 4 had either not entered or were still in the temperature-effective period. This result shows that $V > O$ and $\therefore D < P$ which is certainly not in accord with the interpretation of the

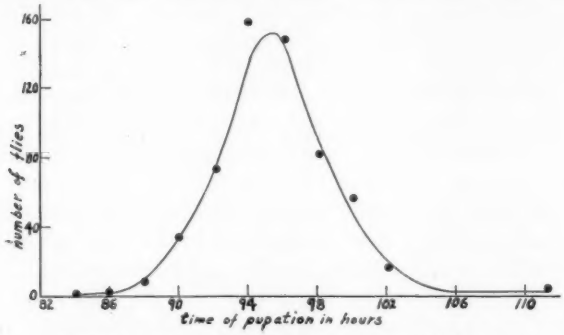


FIGURE 6.—Distribution in time of pupation at 28° ♀ ♀.

temperature-effective period represented by figure 5a. This conclusion, that $V > O$, is further strengthened by the fact that the left-right correlations between flies transferred during their temperature-effective period are greater than zero, while those of flies raised entirely at either temperature are not (table 4). The curves (figures 1–4) obtained in the previous experiments do not, therefore, represent the temperature-effective period in the individual fly.

TABLE 4

Ocellar bristle distribution in ♀ ♀ transferred from 29.7° to 22°.

AGE AT TRANSFER IN HOURS		BRISTLE DISTRIBUTION 2	1	0	NO. OF FLIES	M oc	r(r.1.)
All at 22°		0	0	145	145	0.0	0.0
82	obs.	4	22	126	152	0.10±0.017	0.19±0.078
	calc.	12.6	5.3	134.1			0.82
88	obs.	57	97	98	252	0.42±0.022	0.22±0.06
	calc.	87	36.7	128.3			0.78
93	obs.	301	149	49	499	0.76±0.013	0.19±0.041
	calc.	313	131	55			0.29
All at 29.7°		244	103	18	365	0.81±0.015	0.086±0.051

By further analysis of the left-right correlations of a particular pair of bristles in flies transferred during P, it is possible to show that the interpretation of the temperature-effective period represented by figure 5c is also not in accord with the experimental results. If D = O and V = P, we can calculate the left-right correlation-coefficients of these bristles from the mean bristle number of each transfer group. This has been done for the oc bristles for each of three groups transferred from 29.7° to 22° at different times during the temperature-effective period (table 4). (Calculations made from other bristles and from different experiments yielded similar results.) The calculation is made in the following manner: at any time during P when a transfer is made from T_1 to T_2 there will be, according to this interpretation, two groups of flies, (1) those that will have the mean bristle number and the distribution of 2, 1, and 0 bristles of flies raised entirely at T_1 and (2) those that will have the mean bristle number and the distribution of 2, 1, and 0 bristles of flies raised entirely at T_2. The mean bristle number of the group will be, therefore,

$$M = \frac{n_1 MT_1 + n_2 MT_2}{n_1 + n_2}$$

where n_1 and n_2 represent the number of flies in a transfer group which have not entered and which have passed through their temperature-effective periods, respectively. From this equation n_1 and n_2 can be calculated for each group. Using the distribution of 2, 1, and 0 bristles found among the flies raised entirely at 29.7° and 22°, the distributions in the transfer groups, on the assumption being tested, can be calculated.

$$f(2) = 0.67n_1 + 0.0n_2$$
$$f(1) = 0.28n_1 + 0.0n_2$$
$$f(0) = 0.05n_1 + 1.0n_2.$$

In table 4 these distributions and the left-right correlations calculated from them have been compared with the experimental results. The calculated left-right correlation coefficients are much greater than the experimental ones, showing that the assumption on which they are calculated is incorrect. This result indicates, therefore, that

$$V < P \text{ and } D > O.$$

These experimental data permit only an interpretation of the type symbolized in figure 5b, namely:

$$D > O,\ V > O.$$

A closer approximation to the values of D may be obtained in the following manner: In a group of flies transferred from 29.7° to 22° at the mid-

point of the temperature-effective period of the group at 29.7°, the distribution of flies with 2, 1, and 0 bristles is 57, 97, and 98 respectively and the r(r.1.) = 0.22 (table 4). If P were of type c (figure 5), the distribution would have been 87, 37, and 128 and the r(r.1.) = 0.78 (table 4). The value of D must be such that at the mid-point of P enough flies are in their temperature-effective periods to increase the f(1) at the expense of f(2) and f(0) sufficiently to reduce the left-right correlation coefficient to its observed value. It will be assumed that the frequency distribution of V is normal. The facts that the distribution in time of pupation (figure 6), that the distribution in time of larvae in passing from one instar to another (ALPATOV 1929), and most important, that an experimental determination of the distribution of V (see next section), are apparently normal distributions, justify this assumption.

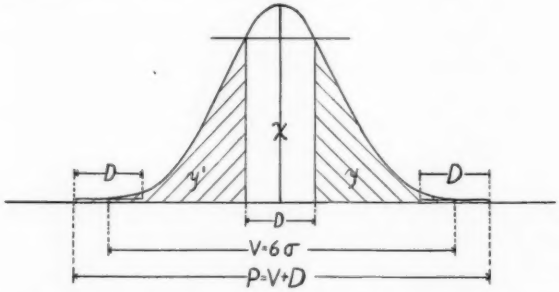


FIGURE 7.—Theoretical distribution of the mid-points of D in a group of flies transferred from T_1 to T_2 at Mv, showing those flies which have passed through, are in and have not yet entered D, y′, x, and y respectively, at the time of transfer.

From a consideration of the number of flies used in this experiment and the form of the probability curve the "practical" range of V will be taken as equal to 6σ. In the population of flies transferred at the mid-point of P (which would also be the mid-point of V), there will be three groups of individuals: (1) those not yet in their temperature-effective period, having the mean bristle number and the distribution of 2, 1, and 0 bristles of flies raised entirely at T_2; (2) those that have completely passed through their temperature-effective period, having the mean bristle number and the distribution of 2, 1, and 0 bristles of flies raised entirely at T_1; and (3) those flies that are in their temperature-effective period, having a mean bristle number somewhere between M T_1 and M T_2, and a distribution of 2, 1, and 0 bristles which is not random (that is, r(r.1.) > 0) since these flies are at different points in their temperature-effective periods. Only those flies the mid-points of whose D's are not farther than D/2 from the mid-point of V (figure 7) (or those within Mv ± D/2 are included in this

third group). These three groups are represented by y, y′, and x respectively (figure 7). (Mv is used to indicate the mid-point of v.)

Since the curve is symmetrical $y = y'$

$$M_{2y} = \frac{M_{29.7°} + M_{22°}}{2} = \frac{0.81 + 0}{2} = 0.405 \text{ (table 4)}$$

$$M_{x+2y} = \frac{xM_x + 2yM_{2y}}{x+2y} = 0.42 \pm 0.02 \text{ (table 4)}$$

$$x + 2y = 252 \text{ (table 4)}$$

$$\therefore x(M_x - 0.405) = (0.42)(252) - (0.405)(252)$$

but since there is no significant difference between M_{2y} and M_{x+2y}, 0.405 and 0.42 respectively,

$$x(M_x - 0.405) = 0$$

but since $x > 0$, $M_x = 0.405$ or 0.42 ± 0.02
as there is no significant difference between these two values.

Among the flies in x

$$f(2) > xM_x^2 \text{ or } x(0.42)^2$$
$$f(1) < 2x(1 - M_x)(M_x) \text{ or } 2x(1 - 0.42)(0.42)$$
$$f(0) > x(1 - M_x)^2 \text{ or } x(1 - 0.42)^2$$

because r(r.1.) > 0.
From the experimental data at 29.7°. Among the y′ flies

$$f(2) = 0.67y'$$
$$f(1) = 0.28y'$$
$$f(0) = 0.05y'.$$

From the experimental data at 22° among the flies in y

$$f(2) = 0$$
$$f(1) = 0$$
$$f(0) = y$$
$$f(2)x + y + y' = f(2)y' + f(2)y + f(2)x$$
$$\therefore 57 > 0.67y' + 0 + x(0.42)^2$$

but

$$x + 2y = 252$$
$$\therefore x > 160$$

or the number of flies in the transfer group, the mid-point of whose D's are within $Mv \pm D/2$ is greater than 160, which is equal to 63.5 percent

of the individuals. From the probability table it is seen that this condition is satisfied when

$$\frac{D}{2} > 0.906\sigma v \text{ or } D > 1.81\sigma v.$$

(σv is used to indicate the standard deviation of v.) To evaluate σv, we may assume that since the temperature-effective period is close to the time of pupation,

$$\frac{\sigma v}{Mv} = \frac{\sigma \text{ pupation}}{M \text{ pupation}} = \frac{3.18}{98.5} \text{ (table 1)}.$$

From table 5 Mv may be estimated as being equal to 88 hours.

$$\therefore \sigma v = 88\frac{3.18}{98.5} = 2.84$$

since $V = 6\sigma v$, $V = 17.2$ hrs.

since $D > 1.81\sigma v$, $D > 5.1$ hrs.

but $P = V + D$

$\therefore P > 17.2 + 5.1$ or 22.3 hrs.

From the data (table 5) P could be anything from 20 to 48 hours because from the experimental data it is rather difficult to exactly determine the limits of P. If those transfer periods, whose mean bristle numbers differ significantly from the means at either temperature, are taken as the time limits of P, P = 20 hours; if those periods whose means show no trend indicative of either the beginning or the end of P are taken as marking the time limits of P, P = 48 hours.

TABLE 5

Temperature effective period 29.7° transferred to 22.0°.

AGE AT TRANSFER, HOURS	NUMBER	MEAN OC	AGE AT TRANSFER, HOURS	NUMBER	MEAN OC
46	189	0.003±0.0028	97	486	0.79±0.013
58	80	0.012±0.0083	102	131	0.72±0.027
72	136	0.018±0.0086	109	91	0.87±0.025
74	97	0.016±0.009	120	83	0.81±0.03
76	563	0.018±0.004	132	77	0.74±0.035
82	152	0.10 ±0.017	144	93	0.86±0.025
88	252	0.42 ±0.022	155	71	0.78±0.033
93	499	0.76 ±0.013	all at 29.7°	365	0.81±0.015

A large error in this latter determination of P may come from the fact that a few delayed eggs may be included among the individuals of an early transfer group producing a spurious mean bristle number for this

group. A greater source of error in determining the limits of P by this method arises in the *subjective estimation* of what points show no "trend," together with the fact that a spurious trend may be obtained when there is a small number of points. These considerations indicate that the latter measure of P = 48 hours may in a sense be a measure of the maximum, just as P = 20 is almost certainly a measure of the minimum value of P.

The conclusions that must be reached from this analysis are: (1) the temperature-effective period of the individual is shorter than that of the population as a whole, (2) there is a variation in the time at which individual flies enter their temperature-effective periods or

$$P = V + D \text{ where } V > 0, D > 0$$

$$D > 1.81\sigma v \tag{3}$$

at 29.7°, D > 5.1 hours, and V = 17.2 hours (on the assumptions made in evaluating σv).

Another approximation to the true value of D has been obtained from experiments of the type suggested by the preliminary experiment mentioned previously (page 141). A group of developing flies were transferred from one temperature to another during P and the larvae removed at two hour intervals as they pupated. The groups of pupae that pupated during the successive two hour intervals were kept in separate vials and the mean bristle numbers of these groups were recorded separately. A number of such experiments were carried out, the transfers being made at various times during P. The results of these experiments are recorded in table 6 for the oc bristles of the females.

These data have been analyzed in a number of ways to determine the maximum and the most probable value of D. From a consideration of the mean bristle numbers and the time at which the flies were transferred from one temperature to another, it can be seen that in no case is there an effect on any flies which pupate within six hours after the time of transfer, and in all instances (save one) the effect of the higher temperature is completed on all flies which pupated twenty hours or more after the time of transfer. (In the experiment where the transfer was made at 82 hours, the effect of temperature is apparently completed only in flies which pupate 28 or more hours after the time of transfer. This experiment was the first of the series to be carried out and it is quite possible that the few (6) flies which make the period for this group much longer than the rest, may have pupated long before they were removed from the bottle and therefore belong to earlier pupation groups.) That is, no fly enters its temperature-effective period earlier than 20 hours before pupation, and every fly has completed its temperature-effective period not later than six hours before pupation. The difference, 14 hours (at 20°) is therefore the maximum value

TABLE 6

Relation between bristle number and time at which flies pupate after being transferred from 28° to 20° during P at time indicated in column heading.

1 82 hours			2 86 hours			3 87.5 hours			4 88.5 hours			5 93 hours			6 96 hours		
t.	No.	Moc	t.	No.	Moc	t.	No.	Moc	t.	No.	Moc	t.	No.	Moc	t.	No.	Moc
82															82	1	1.0
84															84	0	0
86			86	9	0.61	85.5	1	1.0	86.5	13	0.67	85	1	1.0	86	3	0.66
88			88	1	1.0	87.5	2	0.75	88.5	5	1.0	87	3	0.83	88	5	0.7
90			90	0		89.5	4	0.62	90.5	2	0.75	89	4	0.63	90	8	0.5
92			92	3	1.0	91.5	0		92.5	7	0.57	91	8	0.56	92	16	0.63
94			94	3	0.66	93.5	8	0.75	94.5	17	0.56	93	12	0.83	94	32	0.61
96	1	1.0.	96	2	0.75	95.5	24	0.62	96.5	23	0.46	95	18	0.67	96	19	0.58
98	1	0.5	98	8	0.19	97.5	54	0.36	98.5	22	0.16	97	35	0.59	98	10	0.9
100	2	0	100	32	0.14	99.5	80	0.32	100.5	17	0.29	99	20	0.59	100	17	0.50
102	1	0	102	32	0.03	101.5	73	0.16	102.5	9	0.22	101	39	0.36	102	16	0.66
104	13	0	104	45	0.11	103.5	74	0.07	104.5	5	0.10	103	33	0.29	104	14	0.25
106	17	0.06	106	7	0	105.5	79	0.05	106.5	13	0.00	105	33	0.17	106	8	0.37
108	24	0.02	108	9	0	107.5	60	0	108.5	5	0.0	107	23	0.17	108	13	0.15
110	37	0	110	7	0	109.5	53	0	110.5	2	0	109	23	0.09	110	8	0.19
112	30	0	112	5	0	111.5	26	0	112.5	2	0	111	9	0.11	112	1	0
114	44	0	114	7	0	113.5	16	0	114.5	2	0	113	14	0	114	2	0
116	30	0	116	4	0	115.5	14	0				115	0		116	1	0
118	25	0	118	3	0	117						117	0				
120	13	0	120									119	22	0			
122	9	0	122														
124	9	0	124														
126	6	0	126														
128	9	0	128														
130	6	0	130														

of the duration of the temperature-effective period in any fly. Considering each transfer group separately it is seen that the greatest value for the period during which temperature is effective in changing the mean bristle number is 14 hours (columns 1 and 5). These methods both give the same maximum value of D, 14 hours, (at 20°). This value is almost certainly greater than the mean value of D because (1) there must undoubtedly be some variation in the time between the beginning of the temperature-effective period and the time of pupation, and also (2) the class intervals (2 hours) are obviously too large to give an accurate value.

The egg larval periods at 29.7° and 20° are 98.5 and 185.4 hours respectively (table 1). Fourteen hours at 20° is therefore comparable to

$$\frac{98.5}{185.4} \times 14 = 7.4 \text{ hours at } 29.7°.$$

The value of D at 29.7° is, therefore, not greater than 7.4 hours and not less than 5.1 hours (as previously obtained).

From the data (table 6) it is possible to obtain a still closer approximation to the value of D. In table 7 the same data are arranged to show the numbers and percentages of flies of each transfer group that had passed through, were in, or had not yet entered, their temperature-effective periods at the time the transfers were made. These results are shown graphically in figure 8.

TABLE 7

Distribution of flies transferred during P from 28° to 20° grouped to show the numbers of flies which individually have passed through, are in, and have not entered D at the time of transfer.

1	2		3		4	5		
TIME OF TRANSFER, HOURS	PASSED D		IN D		SUM OF COLUMNS 2 AND 3 (FLIES THAT HAVE ENTERED D)	NOT ENTERED D		TOTAL
	NO.	PERCENT	NO.	PERCENT		NO.	PERCENT	
82	2	0.72	94	33.9	34.6	181	65.4	277
86	18	10.2	117	66.1	76.3	42	23.7	177
87.5	39	6.86	360	63.4	70.2	169	29.8	568
88.5	44	30.5	76	52.8	83.3	24	16.7	144
93	111	36.1	160	52.1	88.3	36	11.7	307
96	127	73.0	43	24.7	97.7	4	2.3	174

The lower curve represents the distribution of flies which have passed through their temperature-effective periods and the upper curve shows the distribution of flies that have entered (that is, those that have passed through plus those that are within) their temperature-effective periods. Assuming that, on the average, the first flies to enter the period are the first to pass through it, the most probable value of D may be obtained

in the following manner. From the curves, it is seen, for example, that at 84 hours 50 percent of the flies have entered their temperature-effective periods, and at 94 hours, 50 percent have passed through. The temperature-effective period is, therefore, the difference between these times or 10 hours at 20°, equivalent to 5.3 hours at 29.7°, which value of D is between the maximum and minimum as previously calculated. Other similar calculations made from the curves give similar results, the values of D near the beginning of P, however, being slightly greater than the values of D near the end of P.

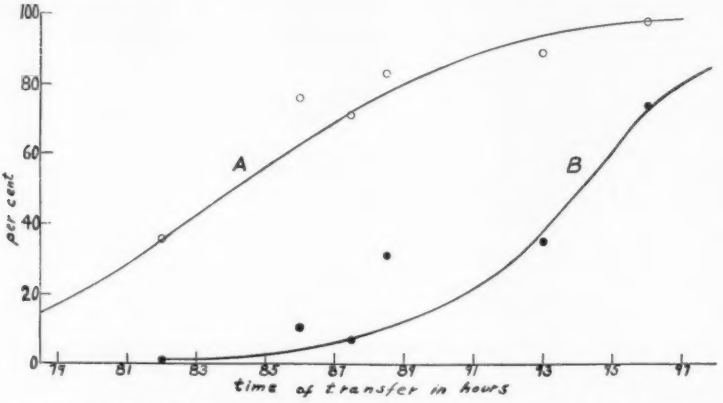


FIGURE 8.—Integral of distribution of V for the beginning and the end of the temperature-effective period. A, percent of flies which have entered D at the time of transfer; B, percent of flies which have passed through D at the time of transfer.

The curves (figure 8) are actually portions of the probability integral curve and it is apparent that they fit the experimental points fairly well thus justifying the assumption of approximately normal distribution of V previously made (page 144) in calculating the minimum value of D. If the data were complete, that is, if they included all the times from the time at which the first flies entered their temperature-effective period to the time at which all the flies have passed through their temperature-effective period, each of these curves would represent the total distribution of V. A rough approximation of the total spread of V can, however, be obtained even from these incomplete curves by doubling the interval of time from the time when half the flies to the time at which all (98 percent in these incomplete curves) the flies have entered the temperature-effective period; or in the second curve, from the time at which the first (1 percent in these incomplete curves) flies have passed through to the time at which all the flies have passed through the temperature-effective

period. These values are 24 hours and 24 hours respectively at 20° or 12.7 hours and 12.7 hours at 29.7°. That these values for V are smaller than that previously obtained (page 146, V = 17.2 hrs.) is due to the fact that these curves are not complete. Both these methods for evaluating V are not satisfactory since the former is based on a pure assumption $\frac{\sigma v}{Mv} = \frac{\sigma \text{ pupation}}{M \text{ pupation}}$ and the latter depends upon "beginning" and end points of V, which are necessarily indefinite.

Another method which circumvents these difficulties has been used to evaluate V from these curves. Since the curves are normal, 1/3 of the individuals on each side of the curve should be contained within $Mv \pm \sigma$. The values of σ so obtained are 5.2 hours and 4.2 hours at 20° for the upper and lower curves respectively. Since the number of flies used in these experiments should be contained within $Mv \pm 3\sigma$, or $V = 6\sigma$, V can be determined. The values of V obtained by this method are 31.2 hours and 25.2 hours at 20° or 16.5 hours and 13.3 hours at 29.7° for the upper and lower curves respectively. That is, at 29.7° the variation in flies entering their temperature-effective periods is 16.5 hours and the variation in flies ending their temperature-effective period is 13.3 hours. The difference between these two may very well be real as there is no reason why the beginning and end of the temperature-effective period should have the same variation, as they are probably determined by different processes. Since P = V + D it follows that D for the earliest flies is somewhat longer than for the latest as noted previously. The variation in the mid-points of D may be taken as the mean of these two values or 14.9 hours instead of 17.2 hours as previously obtained (at 29.7°). The mean value of D was determined as being 5.3 hours (at 29.7°) and therefore the most probable value of P = V + D = 5.3 + 14.9 = 20.2 hours.

$$\therefore \text{ D for the earliest flies} = 20.2 - 13.3 = 6.9 \text{ hours}$$

$$\text{and D for the latest flies} = 20.2 - 16.5 = 3.7 \text{ hours.}$$

P must, however, actually be somewhat longer than this, as it *must* be longer than measured by the method of significant differences. It is perfectly possible, of course, that translation of figures from one temperature to another is not exact, as the correspondence of the temperature-effective periods at different temperatures may not be exact.

The results of the experiments and the analysis of the data on the temperature-effective priod may be summarized thus: the actual duration of the temperature-effective period in any individual fly is not less than 3.8 percent (on the basis of the most probable value of P) and not more than

7.5 percent of the egg-larval period, with a most probable mean value (on the basis of the available data) of 5.4 percent; the temperature-effective period in any one fly lies entirely between the time when 89.3 percent and the time when 96.8 percent of the egg-larval development has been completed. However rough these values may be, they represent the first attempt to determine the actual duration and the temporal location of a temperature-effective period in the individual organisms, all previous determinations giving merely the limits of this period for a group of individuals.

THEORETICAL

In a previous paper (CHILD in press) it was shown that the bristle numbers of the scute fly are markedly affected by temperature whereas those of the wild-type are very slightly affected by temperature. On the basis of the general "rate" theory of development as proposed by GOLDSCHMIDT (1927) and PLUNKETT (1926), this difference in the effect of temperature on the bristle numbers of scute and the wild-type is ascribed to the differences in the effects of temperature on the rates of developmental processes leading to bristle formation in scute and in the wild-type. In scute flies, therefore, the rates or durations, or both, of these developmental processes must differ at different temperatures to produce the observed results. In view of the nature of the experimental material there is no means of determining the rate independently of the duration, whereas the duration of the processes may be independently determined.

The duration of the processes which are differentially affected by temperature is what is called the "temperature-effective period." Such temperature-effective periods have been determined for several characters of *D. melanogaster* by various workers: O. KRAKFA (1924), DRIVER (1931), E. DRIVER (1926, 1931), LUCE (1931), PLUNKETT (1926), STANLEY (1932), and HARNLY (1932). Some of these workers have attempted explanations of the mechanisms of the temperature effect based on the form of the curves obtained by plotting the values of the character measured against the time of transfer. It seems significant that the forms of these curves, in all cases, appear to be sigmoid, resembling the probability integral curve. It has been clearly shown in this paper that the form of such curves, for the bristles of scute-1, is, in fact, determined chiefly by the distribution in time of the temperature-effective periods of the individual flies. It seems, therefore, not unlikely that this may be true also in the other cases cited above. Examination of the data of these workers shows, in fact, that in their cases also the temperature-effective period, as measured, is actually the sum D+V, just as in my data. The interpretations given to their curves by these investigators, namely, that these curves describe the course

of developmental processes leading to bristle, wing, or eye formation, as the case may be, cannot, therefore, be accepted without further analysis of their data from this point of view.

In order to obtain curves actually representing the course of developmental processes, very extensive and accurate data must be accumulated of the kind shown in tables 6 and 7, but with the intervals of temperature transfer and the removal of pupae much shorter. On the basis of the data at present available, no detailed analysis of this kind is profitable.

The end effect of temperature on the bristle number of the fly must be due to an effect on the rate or duration, or both, of the developmental processes leading to bristle formation. We know that the duration of the developmental processes concerned, decreases with an increase in temperature (except above about 29°) and that in all biological systems where the effect of temperature has been studied, an increase in temperature always produces an increase in the rates of the reactions except when temperatures are high enough to be destructive. It is, therefore, possible for an increase in temperature, which decreases the duration and increases the rate, to produce either an increase or a decrease in the end result. It is therefore not necessary to assume, because certain bristles increase and others decrease with temperature, that their developmental processes which are affected by temperature are fundamentally different. These observed differences might be due simply to the relative effects of the temperature on rate and on duration of a common process or processes, being different for the different bristles. Similarly in those cases where a bristle increases with temperature in one part of the range, these results might be due to differences in relative effects of temperature on rates and on durations in different parts of the range.

In these experiments, however, it was found that for all the bristles (with the exception of the anp, when transferred from a lower to a higher temperature, and the orbital bristles) temperature is effective in changing their mean numbers at the same period in development at all temperatures. (Experiments are being performed on the other scute alleles to see whether any generalization can be made with respect to the temperature-effective periods.) This apparent coincidence of the temperature-effective periods for most of the bristles is based entirely on the estimates of the total period $P=(V+D)$. The most probable value of D for the ocellar bristle was determined as being 5.4 percent of the egg-larval period. Small differences in this value of D at different temperatures, not capable of being detected by the rough method used in determining P, could have a profound effect on the mean ocellar bristle number of flies raised at different temperatures. Similarly, small differences in the values of D for the different bristles, although not detectable in the measurement of P,

could produce different mean bristle numbers for the different bristles. The peculiar results obtained with the aor+mor and the anp bristles show still further the complexity of the temperature effect. It is quite apparent from these results that, at least for these bristles, temperature is affecting differentially more than one process in the development of bristles.

There are two considerations which make it seem probable that despite the striking differences in the observed results, the primary effects of temperature on the development of different bristles may be fundamentally similar: (1) the temperature-effective periods for most bristles are the same or nearly the same, and (2) the processes leading to the formation of the different bristles are probably similar, there being no reason to suppose that different bristles should have very different kinds of developmental processes.

The results of these experiments, although they do not critically test the general rate theory of development, are, nevertheless, not in conflict with it. They show the necessity of accumulating large masses of exact quantitative data on developmental processes before definitely formulating and testing a quantitative theory of development.

SUMMARY

1. The temperature-effective period for any particular bristle occupies the same relative portion of the whole egg-larval life at all temperatures.

2. The temperature-effective periods for all bristles (with the exception of the anp, when determined by transferring from a low to a higher temperature, and the aor–mor) occupy the same portion of the whole egg-larval life.

These conclusions (1 and 2) can, of course, be regarded as valid only within the limits of accuracy of the experimental determination of the temperature-effective period.

3. Flies transferred from a lower to a higher temperature during some parts of the temperature-effective period of the anp show a mean number higher than the mean at either temperature.

4. Flies transferred during the temperature-effective period of the aor–mor from one temperature to another, when these two temperatures are on opposite sides of 28°, show a mean which is less than the mean at either temperature.

5. An analysis of the temperature-effective period as measured shows that it is the sum of two quantities, the temperature-effective period of the individual flies and the variation from fly to fly in the time at which their temperature-effective periods are reached.

6. The actual duration of the temperature-effective period of the ocellar bristle in any individual fly is not less than 3.8 percent and not more than

7.5 percent of the egg-larval period, with a most probable value (on the basis of the available data) of 5.4 percent; the temperature-effective period in any one fly lies entirely between the time when 89.3 percent and the time when 96.8 percent of the egg-larval development has been completed.

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STUDIES ON THE BAR SERIES OF DROSOPHILA. I. THE EFFECT OF THE GENE VESTIGIAL ON FACET NUMBER IN BAR

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Received April 23, 1934

A general theory specifying the action of the genes in development was first proposed by GOLDSCHMIDT (1920), as the result of a series of studies on intersexuality in *Lymantria dispar*. The theory, elaborated more fully in 1927, considers the genes as affecting the rates of developmental processes. Since an effect on the rates of some processes limits the durations of others, the varying grades of expression of any character of the organism may be interpreted as the result of some alteration in either the rate or duration of one or more processes in the developmental chain leading from the genes to the character studied. As a formal explanation of the facts, there can be little doubt that the theory is tenable. It lacks, however, any extensive quantitative experimental verification.

Any direct attack upon this problem necessarily involves the possibility of studying directly one or more of the processes in the chain leading from the genes to the adult characters. The difficulties attendant upon such an attempt are apparent. In a form like Drosophila, a direct attack appears impracticable at the present time. We may, however, approach the problem from the standpoint of interaction of genetic factors with each other, and with environmental agents known to affect the expression of various characters of the organism. Since the characters of an organism are the resultants of an interplay of its genes with environmental agents acting upon it throughout the course of development, we may, within certain limits, alter the course of the developmental processes by varying these two kinds of factors either independently or jointly. In working with different genotypes, preferably with biotypes differing only in respect to a single gene, quantitative results relating the character studied to the environmental variables may be obtained. Knowing the functional relations between character and environmental agent for a variety of genotypes, we then have data which may be used for the beginning of an analysis of the action of the gene.

Numerous investigations of this type have been carried out on *Drosophila melanogaster*. A variety of characters which lend themselves to quantitative estimation, for example, facet number, wing area, bristle number, and others, have been studied in different mutant stocks. For the most part, temperature was the environmental agent used in the experiments. In an investigation on bristle number in Dichaete flies, PLUNK-

ETT (1926) analyzed his data in terms of conventional chemical kinetic concepts. In virtue of its peculiar temperature characteristic PLUNKETT was able to single out a "bristle-destroying" reaction which simulated the kinetics of an irreversible unimolecular reaction. This reaction was identified as the destruction of a thermolabile bristle-forming catalyst. An exhaustive quantitative analysis of the data showed that the relations observed were, within the limits of experimental precision, in excellent agreement with results predictable from theory. PLUNKETT concluded that the difference between Dichaete and wild-type, in respect to bristle production, was attributable to an acceleration of the above reaction in Dichaete flies. We find in PLUNKETT'S work the first attempt at application of a simple kinetic theory to data amenable to quantitative treatment. These results are especially interesting since they led PLUNKETT, quite independently, to the same conclusions as GOLDSCHMIDT in respect to the action of the genes in development.

The Bar series of alleles in Drosophila, have been investigated more than any other genes in relation to the effect of temperature on a character susceptible to quantitative estimation, in this case, facet numbers. These studies have afforded investigators a considerable body of data, both on the facet-temperature relation and on the duration of the temperature-effective period, for a whole series of alleles. A number of explanations of the action of the Bar gene in relation to facet production have been proposed. For the most part, these explanations serve adequately as formal explanations of the action of the Bar gene for a limited group of data. None, however, is consistent with the whole body of data on facet number in the Bar series, now available. This question will be considered more fully, later.

In the investigation here presented, the effect of the gene, vestigial (*vg*), on facet number in Bar-eyed (*B*) flies was studied. Apart from its conspicuous effect in reducing the size of the wing, *vg* also effects an increase in the duration of development, especially in the egg-larval period. Since an increase in the total time of development may to some extent alter the relative durations of various developmental processes, *vg* may be expected to affect the somatic expression of a variety of characters in some degree.

In addition to the major problem considered, a preliminary investigation on wing area in *vg* and *Bvg* flies at 27°, showed no significant difference. Although this result does not preclude a possible effect of *B* on wing area in *vg* at other temperatures, this phase of the problem was not continued. The plan of experimental procedure in determining the effect of *vg* on facet number in *B* is the following:

1. A comparison of facet numbers in *B* and *Bvg* flies over a range of temperatures.

2. A determination of the length of the period during which temperature is effective in determining facet number in *B* and *Bvg* flies.

The results of the first phase of the investigation are presented here. The results on the temperature-effective period investigation will be considered separately.

EXPERIMENTAL

Materials and Methods

Experimental Stocks

Three mutant races of flies were studied: (1) *B*, (2) *vg*, and (3) *Bvg*. These three groups were rendered isogenic for possible modifiers through inbreeding by pair brother to sister matings over a period of 14 months before the experiments were begun, and involving some 30 to 35 generations. The system of inbreeding used was the following:

P_1. $B\,♂ \times vg\,♀$
P_2. Inbreeding of F_1 sibs
P_3. $B\,♂ \times vg\,♀$ (obtained from F_2)

In this way the cycle of inbreeding was repeated every two generations. Throughout the inbreeding, selection for low facet number and general vigor was practiced. Unfortunately, selection for the latter was not successful, as the stocks which were finally obtained showed very low fertility and fecundity, and poor viability as evidenced by a fairly high mortality in pupal stages. This was especially true of the *vg* and *Bvg* stock, and necessitated the use of large numbers of flies in all the experiments where short egg-laying periods were required.

Thirty generations of inbreeding should insure a high degree of genetic uniformity in the stocks. An attempt was made to test this point by use of the parent-offspring correlation, in respect to facet number. This proved unsuccessful at first due to the difficulties attendant upon the use of pair matings in these stocks. A second parent-offspring correlation was tried on the *B* stock, a year after inbreeding had been discontinued. This time a sufficient number of offspring was obtained from 20 pairs of parents to make the test possible. The value of the coefficient for male and female respectively was found to be:

$$r♂ = 0.17 \pm 0.067,\ n = 210$$
$$r♀ = 0.25 \pm 0.065,\ n = 210$$

These values are quite small but statistically significant, although the small number of parents on which the values are based, leaves the question of a real correlation open to doubt. However, certain other data indicate that the correlation may be real. In table 1, are given the values for facet

counts on *B* at 28°, for a series of six experiments. This temperature was used as a control throughout the investigation. The measure of sampling errors used throughout the investigation is the standard error, $\frac{\sigma}{\sqrt{N}}$. The individual means scatter quite at random about the grand mean, and for the most part show no significant deviations. The largest difference occurs in the males of experiment 5. Here the deviation is equal to 4.2 times its standard error. This difference, however, does not represent a single difference between two samples, but rather the largest deviation in a group of 12. Here the simpler tests of significance cannot be used. For a group of ten means, a single deviation as great as 3.2 times its standard error lies on the 5 percent level of significance (TIPPET 1931).

TABLE 1

Facet counts at 28° Bar.

EXPERIMENT	M	σ	v	n	FACET RANGE
		MALES			
1	51.7±0.69	7.14	13.8%	106	39–68
2	49.9±0.80	5.70	11.4%	51	41–67
3	49.2±0.95	5.48	11.2%	33	40–59
4	52.0±0.48	5.80	11.2%	146	39–74
5	54.3±0.78	6.20	11.4%	64	41–70
6	47.7±0.69	7.46	15.9%	119	31–77
Mean	50.8±0.29	6.52	12.8%	519	
		FEMALES			
1	46.1±0.88	8.0	17.4%	85	33–68
2	48.6±0.96	5.9	12.2%	38	37–63
3	49.8±1.20	6.7	13.4%	32	37–63
4	51.9±0.50	5.5	10.6%	119	38–68
5	51.9±0.94	6.2	11.9%	44	38–64
6	48.3±0.72	7.2	14.9%	101	35–67
Mean	49.4±0.33	6.69	13.5%	419	

According to this criterion the deviation under consideration still appears significant. However, considering the fact that the mean for the females in experiment 5 shows no significant departure from the grand mean, we must conclude that we are probably dealing solely with sampling errors. A comparison of the range of the facet counts in table 1 shows in general no trend, with the exception of the males in experiment 6. The ranges of the facet counts are quite uniform in the different experiments. This constancy is more strikingly illustrated by the coefficients of variability (v). Neglecting the data for experiment 6, since the results of this experiment are not included in this paper, it seems clear that no perceptible amount of mutation affecting facet number had occurred during the course of the experiments. If, then, the parent-offspring correlations found are

real, some slight genetic heterogeneity must have been present in the stocks at the completion of inbreeding.

The question may be considered from another point of view. PLUNKETT (1926) pointed out that the absence of a right-left correlation for any character indicated a complete uniformity of all factors affecting that character in the population studied. In the bristles which he was studying, no right-left correlations appeared, showing that all factors which might affect bristles were acting uniformly on all individuals. In table 2, right-left correlations for facet number at a series of temperatures are tabulated.

TABLE 2

Left-right correlations for facet number.

STOCK	T	r	n	M (left)	M (right)
		MALES			
B	20°	0.776±0.080	49	123.9±2.52	123.9±2.66
B	22°	0.677±0.077	49	105.2±2.34	106.0±2.40
B	25°	0.678±0.073	51	78.8±1.19	77.2±1.19
B	27°	0.583±0.067	98	58.9±0.72	58.5±0.70
B	27.8°	0.570±0.060	146	52.1±0.52	52.0±0.48
Bvg	22°	0.669±0.088	39	102.8±2.19	104.2±2.23
		FEMALES			
B	20°	0.721±0.070	47	111.2±2.29	113.0±2.17
B	22°	0.577±0.096	48	95.4±2.00	98.7±2.11
B	25°	0.643±0.088	45	76.3±1.66	75.3±1.48
B	27°	0.437±0.100	65	55.9±0.87	56.0±0.92
B	27.8°	0.596±0.059	119	52.2±0.48	51.9±0.50
Bvg	22°	0.828±0.042	55	91.1±2.22	91.3±2.21

At all temperatures the correlations are high. Assuming that the parent-offspring correlations found are real, we should then expect some right-left eye correlation. Precisely what the right-left correlations should be for varying magnitudes of the parent-offspring correlation, is not apparent. An analytical investigation of this question has not been undertaken. In this connection, however, certain data of PLUNKETT (1926) are interesting. In his "*D6*" stock which showed a parent-offspring correlation of 0.412, PLUNKETT found that the right-left correlations between four different bristle pairs were of approximately the same magnitude as the parent-offspring correlation. The mean of the four values was 0.386. It appears then that the right-left correlations on facet number found here are too high to be accounted for by slight genetic heterogeneity, or by differential action of environmental agents on individual flies. In this event the high right-left correlations found would in some measure be due to another factor or factors.

It has been shown that, in general, various parts in Drosophila develop

independently. The evidence for this comes largely from the work of REITH (1925), and from the many observations on localized effects of the genotype in the development of various parts in mosaics. It is, however, entirely possible that some structures may show some degree of interdependence in development. There is evidence for this in the case of vermilion eye color (STURTEVANT 1920 b). Moreover, in Dichaete, PLUNKETT (1926) found definite associations between certain bristles on the same side of the fly. These associations clearly indicate some type of unilateral developmental dependence.

A mechanism which would explain the interdependence of the two eyes may be formally presented by the assumption that certain substances entering into the formation of the eye are elaborated medially at some early developmental stage, and then distributed to each side. A condition of this type is entirely possible in Drosophila, where the primordium of the optic discs appears at first as a single structure located medially and later gives rise to the two frontal sacs which develop into the optic discs (CHEN 1929). The earliest appearance of the optic primordium in Drosophila was not actually observed by CHEN, but was established by analogy with Rhagoletis (SNODGRASS 1924). Further speculation on this point would not be profitable since no critical evidence is at present available. It should be pointed out, however, that although the absence of any right-left correlation definitely indicates uniformity of both genetic and environmental factors affecting the character in question, the presence of a correlation does not necessarily imply a lack of uniformity of these same factors.

Methods

The culture medium used in all experiments was the usual banana-agar preparation, consisting of equal parts of water and banana, and 2 percent by weight of the total of agar-agar. An effort was made to secure bananas of the same degree of ripeness for all experiments. Four by one inch shell vials were used in all the experiments. Approximately 15 cc of food was placed in each vial and then inoculated with a drop of a thick yeast suspension (one cake of yeast to 100 cc of water). The food was never used until the day following its preparation, in order to insure an adequate yeast growth. Ten pairs of flies per vial were permitted to lay eggs over a period of two hours. It should be pointed out that the use of a large number of flies for egg-laying tends to minimize any differential effects resulting from genetic differences which may be present in the individual flies. Flies of 4 to 5 days of age were used for egg-laying as this is very near the optimum age for egg-production. To avoid possible crowding effects only vials in which less than 25–30 flies developed were used. LUCE (1931)

reported that crowding causes a marked diminution in facet number in infra-bar, an allele of Bar. In my own experiments, a parallel, though much smaller effect, was found. As a rule, less than 50 flies developing per vial does not produce any decrease in facet numbers in Bar.

All the temperature experiments were carried out in incubators in which temperature variations were limited to a range of $\pm 0.1°$. For most temperatures the variation was much less. In the few cases in which temperature fluctuation was known to be of a magnitude sufficient to account for aberrant results, the experiments were discarded.

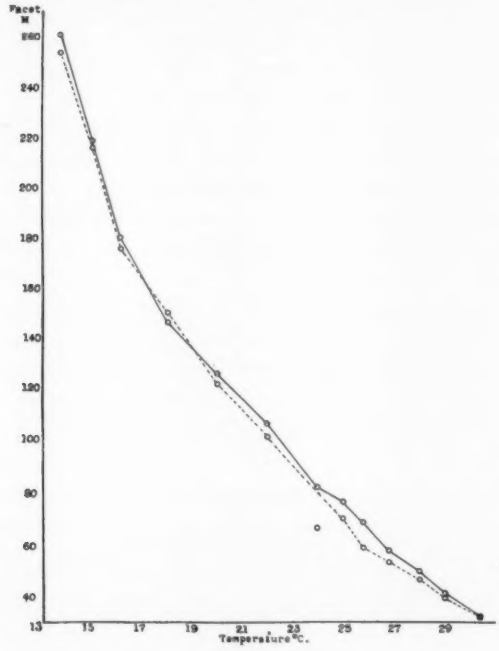


FIGURE 1
The facet-temperature relation (♂♂)
B—solid line
Bvg—broken line

At most temperatures, facets were counted directly under a Bausch and Lomb dissecting microscope, using a 17× ocular and 25 mm objective. At 13.7°, 15°, and 16°, dissections of the cornea were made and the facets counted on a projecting apparatus, as the eyes were too large to count directly. Error in counting was checked by making recounts of a number of flies at different temperatures. In all cases the personal error was somewhat less than 1 percent.

Temperature Experiments

Effect of Temperature on Facet Number

The effect of temperature on facet number in *B* and *Bvg* was studied at 1° or 2° intervals over the complete range of temperatures in which the flies are able to develop. The essential statistics on facet number at different temperatures are presented in table 3. The data are recorded separately for males and females, since there is definite sexual dimorphism for facet number as, in fact, for most other characters of Drosophila which have been studied quantitatively. In figures 1 and 2, the facet-temperature

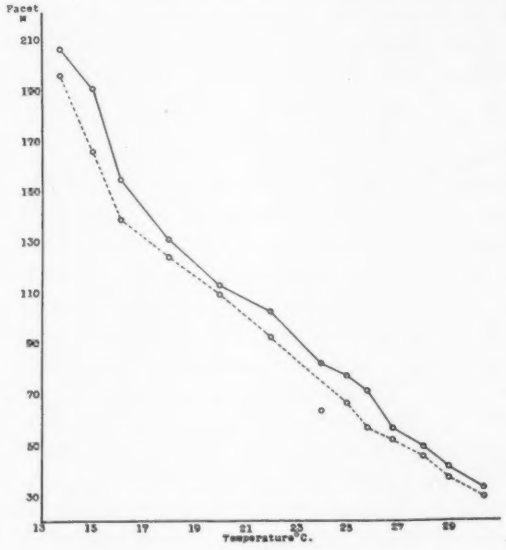


FIGURE 2
The facet-temperature relation (♀ ♀)
B—solid line
Bvg—broken line

relation is shown graphically. The effect of *vg* on facet number in *B* is apparent. At all temperatures (with exception of *Bvg* males at 18°) *vg* effects a small but consistent reduction of facet numbers. The effect is somewhat greater in the females than in the males. In the males the facet differences between *B* and *Bvg* are not statistically significant at temperatures below 22° or at 30.4°, but with one exception noted above, the differences are always in the same direction. The facet differences between *B* and *Bvg* in the females are not statistically significant at 13.7° or at 20°, but are always in the same direction. It must be concluded that the effect

of *vg* on facet number in *B*, though small, is real. MAY (1917) in selection experiments on facet number in *B* and *Bvg* flies, observed that, in general, facet number was slightly lower in *Bvg*. Since his stocks were not isogenic with each other, and temperature was inadequately controlled, his observations can hardly be considered as supporting the results presented here, though they are quite in agreement. The reason for the departure of *Bvg* ♂♂ and ♀♀ at 24° from the general trend of the curves is not clear. Some experimental error is probably involved.

The facet-temperature relation in the different members of the Bar series has been extensively investigated. SEYSTER (1919), KRAFKA (1920), and DRIVER (1931) have all observed an inverse relation between temperature and facet number. There are, of course, differences in the form of the curves obtained by these investigators. These, no doubt, are due to genetic differences in the stocks used. The temperature-facet relation presented here for *B*, conforms most nearly to that found by KRAFKA. LUCE (1926) found that infra-bar, an allele of *B*, behaves quite differently in respect to temperature. Facet number increases in infra-bar with increase in temperature, showing a maximum between 27° and 30°, and then decreases. This fact is pointed out here since it is of considerable importance in any interpretation of the action of the *B* gene, as will be shown later.

The statistics other than the mean in table 3 are of some general interest. The coefficient of variability (v) which we have taken as σ/M expressed as a percent, is a good measure of the relative variability of a population. The values of v (table 3) for male and female show no systematic differences. This is true, also, for *B* as compared with *Bvg*. Moreover, there is no indication of a trend in v with temperature. In respect to this last point, these data differ from those of KRAFKA (1920) who finds a systematic increase in v with increase in temperature. On the other hand, DRIVER'S data (1931) show that v is constant at all temperatures except 30°. The relative constancy of v for facet number in the data of table 3 is emphasized here, since it bears upon certain considerations in the investigation on the temperature-effective period.

Duration of Egg-Larval Period

The criterion used in determining the end of the larval period was the eversion of the anterior spiracles. Individuals which had completed the egg-larval period were transferred to agar slants at two hour intervals to complete their development. At 16.1°, a four hour interval between observations was used. The sex ratios were determined at the time of emergence from the pupa cases.

The data on duration of the egg-larval period for *B* and *Bvg* are presented in table 4. The general relation between temperature and length

TABLE 3

		B				*Bvg*			
T	M	σ	v	n	M	σ	v	n
				MALES				
13.7°	260.7±4.4	32.4	12.4%	55	253.2±9.1	43.7	17.3%	23
15°	218.8±5.1	32.3	14.8%	41	215.9±4.1	24.2	11.2%	35
16.1°	180.0±2.7	26.9	14.9%	97	175.5±4.1	22.3	12.7%	30
18°	146.6±3.5	22.7	15.5%	43	150.4±4.6	26.7	17.8%	33
20°	126.9±3.2	19.1	15.1%	35	122.5±2.8	14.3	11.7%	30
22°	107.8±1.4	18.3	17.0%	176	102.4±1.4	14.8	14.5%	106
24°	84.0±1.3	11.4	13.6%	74	68.1±1.5	8.3	12.2%	30
25°	78.2±1.2	10.3	13.2%	75	71.7±1.3	7.5	10.5%	33
25.8°	70.1±1.8	11.4	16.3%	78	60.2±1.1	7.1	11.8%	39
26.8°	58.9±0.6	7.5	12.7%	143	54.4±0.8	5.7	10.5%	55
28°	50.8±0.3	6.5	12.8%	519	47.1±0.6	6.0	12.7%	117
29°	41.8±0.5	3.7	8.9%	48	39.9±0.7	3.8	9.4%	31
30.4°	32.7±0.9	4.4	13.3%	26	32.4±0.8	3.0	9.3%	14
				FEMALES				
13.7°	205.8±3.9	28.9	14.0%	56	195.7±7.8	39.9	20.4%	26
15°	190.5±5.3	28.3	14.9%	29	165.7±4.5	18.6	11.2%	17
16.1°	154.4±2.3	22.4	14.8%	94	138.4±2.9	15.6	11.3%	28
18°	130.6±3.2	17.0	13.0%	28	123.7±3.3	19.0	15.4%	33
20°	112.8±2.2	16.4	14.5%	55	109.1±2.3	14.0	12.9%	36
22°	102.4±1.2	15.1	14.7%	150	92.4±1.4	15.7	17.0%	119
24°	82.1±1.1	12.3	15.0%	120	63.2±0.7	5.7	9.0%	64
25°	77.2±1.3	10.6	13.7%	72	66.1±1.1	6.8	10.3%	40
25.8°	70.9±1.6	11.2	15.8%	47	56.5±1.1	5.9	10.4%	28
26.8°	56.3±0.7	7.5	13.3%	106	52.0±0.8	6.1	11.7%	55
28°	49.4±0.3	6.7	13.5%	419	45.3±0.6	5.2	11.5%	86
29°	41.2±0.5	3.6	8.8%	61	37.0±0.7	4.2	11.4%	36
30.4°	32.9±0.6	3.5	10.5%	35	29.3±0.5	2.7	9.2%	26

TABLE 4

Duration of egg-larval period.

				B				
	MALES				FEMALES			
T	M	σ	v	n	M	σ	v	n
16.1°	297.3±1.40	11.6	3.9%	69	297.1±1.57	13.5	4.5%	74
22.0°	143.6±0.38	6.0	4.2%	247	142.6±0.33	4.9	3.4%	212
27.0°	97.7±0.50	3.6	3.7%	53	97.2±0.53	3.6	3.7%	47
28.0°	95.4±0.44	3.1	3.2%	49	95.6±0.72	4.5	4.7%	39
29.0°	93.9±0.66	3.8	4.0%	35	95.5±0.52	3.2	3.4%	39
*30.2°	98.8±0.21	2.6	2.6%	176				
				Bvg				
*22°	150.3±0.98	8.1	5.4%	68				
27°	101.9±0.66	4.2	4.1%	41	102.2±0.63	3.9	3.9%	39
28°	97.7±0.57	3.4	3.5%	36	98.0±0.98	4.7	4.8%	23
29°	96.7±0.52	3.0	3.1%	33	96.6±0.68	3.4	3.5%	26

* Males and females were not recorded separately.

of the egg-larval period is the same as that found by other investigators. Time of development decreases with increase in temperature, with a minimum at about 29°. Beyond this point the time increases slightly. It may be seen, further, that *vg* increases the time for egg-larval development. HARNLY (1929) found that *vg* increased the duration of the egg-larval period about 5.5 hours at 25°C. He found, also, that there is little, if any, effect of *vg* on the pupal period. Although no data for 25° are available in table 4, the values at 22° and 27° for *B* and *Bvg* are of a magnitude entirely consistent with HARNLY's results.

No sexual difference in duration of the egg-larval period was found at any temperature in the present experiment. In this respect the data confirm the observations of LUCE (1931) on infra-bar. They differ, however, from the observations of other investigators. POWSNER (unpublished), and CHILD (1935) working in this laboratory on Drosophila stocks unrelated to each other or to mine, found that at all temperatures the males have a shorter egg-larval period than the females. On the other hand, BONNIER (1926) observed that at 30° the egg-larval period for the females was shorter, but found no sexual difference at 25°. The disparity in the results of different investigations is, no doubt, attributable to genetic differences in the stocks.

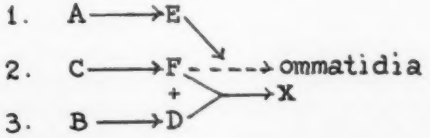
Discussion

Any interpretation of the effect of *vg* on facet number in *B*, necessitates first a consideration of the action of the Bar gene itself in facet production. From the observed facts on facet number in *B*, at least two simple possibilities are indicated. (1) We may visualize the processes initiated by *B* as forming part of the general facet-producing system, but resulting in a slower production of facets. (2) Alternatively, *B* may initiate processes which act entirely independently of the facet-producing mechanism. These processes may act in a manner which results in the "destruction" of facet-forming material. Either of these systems equally well explains the facet decrease in the presence of *B* or any of its alleles. Moreover, either system can be adjusted to explain the facet temperature relations observed in the *B* series. However, a consideration of the possible effects of factors which may alter the duration of the facet-determining processes, shows a significant difference in the two systems. According to (1) an increase in the duration of the processes would effect an increase in facet number, while in (2) an increase in duration would result in a decrease. As will be shown, (2) is consistent with all the known facts relating to the Bar series, and lends itself, moreover, to an interpretation of the results presented here.

KRAFKA (1920) explained the facet-temperature relation in *B* on the basis of two independent sets of processes occurring in facet determination.

One is a facet-producing system, and the other a facet-reducing system. The facet number which develops in *B* is then the resultant of these two sets of processes. In explaining the temperature effect on facet number, it was assumed that the temperature coefficient of the second set of processes was greater than that of the first. This explanation was entirely in accord with the facts then known. It does not, however, take into consideration the question of duration of the processes involved in facet formation. ZELENY (1923) in discussing the effect of temperature on facet number, states, "The change in ommatidial number with change in temperature can be explained most satisfactorily by assuming a differential effect of temperature upon the physiological processes involved in ommatidial production as opposed to other physiological processes. In view of the fact that temperature is effective only during a few hours of larval life, it may be considered that the initial steps in the formation of ommatidia are confined to a definite embryological period. The length of this period is determined by the general physiological processes of the larva, while the rate of formation of ommatidia during the period is a function of special processes which have a different coefficient. It is evident that under these circumstances two different temperatures must give two different ommatidial numbers."

The following simple system formally illustrates the relations of the two sets of processes postulated by KRAFKA, and in addition the role of factors which govern the duration of these processes.



In the above diagram we have three independent sets of processes, which we may consider as irreversible reactions. We are not at the moment, concerned with the exact nature of the three sets of reactions illustrated, but rather with their interrelations. The production of substances E, F, and D, from A, C, and B, respectively, is purely schematic. Quite probably each reaction would be more accurately represented by a reaction chain. Reaction (2) represents the facet-forming processes in the wild-type, resulting in the production of F, whose quantity at a particular time determines the number of facets. The assumption that this reaction occurs in the Bar series is not gratuitous. The work of BRIDGES (1917), and of STURTEVANT (1925), shows that the normal allele of B plays no part in facet production, and that consequently other genetic factors are concerned. The broken line from F to ommatidia denotes a morphological transforma-

tion of a region containing F into facets. This transformation, based upon the facts of ontogeny, occurs "instantaneously" in the sense that all facets are visibly differentiated at the same time. Reaction (3) is not present in the wild-type, but is found in members of the Bar series. This reaction initiated by *B* leads to the formation of a substance D which combines with F to form X. It should be pointed out that for our present purpose D may equally well be considered as a catalyst, catalyzing the reaction F→X. The combination of F and D is the "facet-destroying" process previously mentioned. We are not concerned with the nature of X, except to note that its production effects a diminution in F. In considering the time relations of reactions (2) and (3), we may assume that F is produced at some early stage of development, and in sufficient quantity to give the facet number of the wild-type. Reaction (3), then, occurs somewhat later so that F combines with D as the latter is being formed. It should be pointed out that the time relations for the reactions here presented are not essential to the general theory, and can only be established by other experimental data. If the conditions postulated are correct, the time-facet relation will be sigmoid in character.

Reaction (1), resulting in the formation of a substance E, represents really the aggregate of all developmental processes which determine the time at which the region containing F is converted into facets. These processes occur, of course, in all stocks, but will vary considerably according to the genetic constitution of the stocks.

It is of interest to note what factors limit the duration of the reaction F+D→X. We have assumed that F is produced and localized at some early developmental period, that is, prior to the beginning of reaction (3). Consequently, the reaction F+D→X begins when D is produced and begins to combine with F. The factors determining the end of the reaction are, as we have seen, entirely independent of D and F, but depend rather on a great many other processes of general development which determine when differentiation of the optic disc into ommatidia may take place. This obviously implies that the reaction F+D→X need not go to completion. PLUNKETT (1932) has pointed out that very probably a great many developmental reactions controlled by mutant genes do not actually go to completion, but may be terminated by extrinsic factors.

The facet-temperature relations observed in the Bar series may now be considered in relation to the system of reactions postulated. If the temperature coefficient for reaction (1) is less than that for the formation of X, an increase in temperature will produce a decrease in facet number. This relation is observed in *B* and *BB*. If, on the other hand, the temperature coefficient for reaction (1) is greater than that for the formation

of X, facet number will increase with increase in temperature. This is the condition found in B^i and B^iB^i over the greater portion of the temperature range in which the flies are viable. LUCE'S observation that B^i shows a maximum facet number between 27° and 30° may very well be related to the fact that time of development increases at temperatures above 29°. As a consequence, in B^i the "facet-destroying" reaction (F+D→X) should take place over a relatively longer period of time at high temperatures, and result in a decrease of facet number with increase in temperature, above 29°.

The effect of *vg* on facet number in *B* may now be interpreted. It has been shown that *vg* increases the length of the egg-larval period. Since a retardation in the rate of general development will to some extent retard the rates of those developmental processes which determine the time at which F is converted to facets, we should expect the reaction F+D→X to take place over a somewhat longer period of time in *Bvg* than in *B*. This, clearly, will result in a diminution of facet number in *Bvg* as compared with *B*, a fact already observed. It seems quite probable that crowding acts in this same way, that is, by retarding development.

It is, of course, desirable to test more directly the effect of *vg* on the duration of the reaction F+D→X. The work of KRAFKA (1920), and especially that of DRIVER (1931), and LUCE (1931) has demonstrated that the effect of temperature on facet determination in the Bar series is restricted to a definite portion of larval life. Moreover, KRAFKA (1924) and CHEN (1929) have shown that the end of the temperature-effective period corresponds very closely to the time at which ommatidia are differentiated as discrete units. These results make possible an identification of the reaction F+D→X with the temperature-effective period. We cannot, of course, dismiss the possibility that other reactions involved in facet formation are also affected by temperature. The results of R. K. HERSH (1924) on the temperature-facet relation in the wild-type fly show such an effect of temperature, although much smaller than that found in the Bar series. Speculation on this point is hardly profitable since the validity of the above identification rests to some extent on the correctness of the time relations of the different facet-determining processes here postulated. For this purpose a knowledge of the temperature-effective period for facet determination in the wild-type fly is essential.

If the identification of the reaction F+D→X with the temperature-effective period is correct, then we may test the interpretation given here of the effect of *vg* on facet number in Bar, by determining the length of the temperature-effective period in *B* and *Bvg*. The results of this phase of the investigation will be submitted in a separate communication.

SUMMARY

1. An investigation of facet numbers in *B* and *Bvg* flies over a range of temperatures, showed that *vg* effected a small but significant decrease in facet number.

2. Three sets of reactions which formally explain the facet-temperature relations observed in the Bar series are postulated. In this system of reactions, the essential difference between wild-type and Bar is the presence in the latter of a "facet-destroying" reaction, $F+D \rightarrow X$, in which F represents facet-forming material, and D a substance whose formation is initiated by the Bar gene.

3. The effect of *vg* on facet number in *B* is interpreted as resulting from an increase in duration of the reaction, $F+D \rightarrow X$, thereby decreasing the quantity of F available for facet formation.

ACKNOWLEDGMENT

The writer is greatly indebted to Dr. C. R. PLUNKETT for his helpful guidance and supervision throughout the investigation.

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SOME OBSERVATIONS ON THE STRUCTURE OF THE SCUTE-8 CHROMOSOME OF DROSOPHILA MELANOGASTER

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Received April 23, 1934

STRUCTURE OF THE SCUTE-8 CHROMOSOME

We have been interested in the structure of the X chromosome of the stock known as scute-8 apricot, found by SIDOROV (1931). This stock has been used extensively in our laboratory, and it early became a matter of importance to have exact information on the nature of this chromosome. It was of course known that it contained a long inversion, but its exact limits had not been fully worked out. The fact that the locus of bobbed was inverted was shown in 1932 (PATTERSON 1932b, p. 154), and a diagram of the scute-8 apricot chromosome, based on all of the evidence then available, was published in 1933 (PATTERSON 1933, p. 34).

In this diagram it was indicated that the inverted section extends from a point lying just to the right of the locus of scute (scute-8), to some point lying between the locus of bobbed and the fiber-bearing end of the chromosome. The genes in the inverted section appear in reverse order, with the locus of bobbed lying near to that of yellow, and followed by the loci of such genes as carnation, forked, garnet, and so on, to white, finally ending with the so-called gene for viability (PATTERSON 1932a). We now have evidence which shows that the left-hand break occurred between achaete and scute, so that the locus of scute-8 is also inverted.

In a paper dealing with mosaic formation (PATTERSON 1933) experiments were described in which scute-8 apricot flies had been X-rayed and mated to untreated yellow white crossveinless miniature forked-5 flies. Among the F_1 flies were found 146 yellow apricot hypoploid females, the so-called aberrant females. These were interpreted as having resulted from the breaking off and elimination of the left end of the treated scute-8 chromosome, thus creating a deficiency at the locus of yellow.

If the locus of bobbed is inverted and lies near to that of yellow, then a certain percentage of these yellow aberrant females should also be deficient at the bobbed locus. In order to determine this point, 61 of these yellow females were tested to echinus forked bobbed males. The results show that eight of the 61 were also deficient for bobbed, as indicated by the appearance of bobbed females among their offspring. The conclusion to be drawn from this test is that the locus of bobbed lies near to that of yellow, and is therefore involved in the inversion.

The fact that 53 of the 61 females tested to bobbed showed that the

break had taken place between the loci of yellow and bobbed, and that only 8 showed that the break had occurred to the right of the bobbed locus, would indicate that there must be present between yellow and bobbed a considerable piece of the "inert region" of the X chromosome.

THE POSITION OF THE SCUTE-8 LOCUS

We may now consider the genetic evidence which has a bearing on the locus of the scute-8 gene in the inverted section. As stated above, it was formerly assumed that this locus was not inverted, but lay at its original position, just to the right of yellow. The first evidence that this might not be the case was obtained in an experiment in which treated scute-8 apricot males were mated to females having attached X chromosomes, homozy-

TABLE 1

Results of crosses between sc^1 f car bb males and F_1 v g f females, which were derived from X-rayed sc^8 wa males mated to females with attached XX's, and homozygous for y v g f.

CASE NUMBER	SEGREGATION TYPES		NON-DISJUNCTION TYPES	
	y v g f FEMALES	HYPERPLOID MALES	*v g f* FEMALES	*sc^1 f car bb* MALES
606j	6	1, $\frac{sc^1}{sc^8}$ *f car bb*	1	0
600k	2	4, $\frac{sc^1}{sc^8}$ *f car*	0	0
601c		8, $\frac{sc^1}{sc^8}$ *f car*	0	0
603f	12	3, $\frac{sc^1}{sc^8}$ *f car*	0	0
605a	13	2, $\frac{sc^1}{sc^8}$ *f car*	0	0
605g		3, $\frac{sc^1}{sc^8}$ *f car*	0	0
605m	14	7, $\frac{sc^1}{sc^8}$ *f car*	0	0
606i	17	7, $\frac{sc^1}{sc^8}$ *f car*	0	0
603a	1	1, $\frac{sc^1}{sc^8}$ *f*	2	0
606b	11	3, $\frac{sc^1}{sc^8}$ *f*	2	2
606e	5	6, $\frac{sc^1}{sc^8}$	0	0
603c	6	3, $sc^1 f$	0	0
606q	9	3, $sc^1 f$	1	0

gous for yellow vermilion garnet forked. A number of F_1 females carrying a duplication was obtained from this cross. Such females were non-yellow vermilion garnet forked. They had been produced by a long deletion eliminating the middle part of the X chromosome. These F_1 females were tested to scute-1 forked carnation bobbed males. In thirteen cases the hyperploid males were found to be viable, and this gives an opportunity to determine more exactly where the two breaks in the chromosome, responsible for the deletion, had taken place. These thirteen cases are listed in table 1.

The results obtained in the first eleven cases do not give any decisive evidence with reference to the position of scute-8; it could as well lie at the left as at the right end of the inverted section. But they do show where the left-hand break had taken place. Thus in the first case (606j), the single hyperploid male reveals the "compound" of scute-1 plus scute-8, and the mutant characters forked, carnation, and bobbed. The left-hand break therefore occurred at some point lying between yellow and bobbed. In the next seven cases (600k, 601c, 603f, 605a, 605g, 605m, 606i) the hyperploid males reveal the compound of scute-1 plus scute-8, and the mutant characters forked and carnation. Here the left-hand break in each case must have taken place between the loci of carnation and bobbed. In the next three cases (603a, 606b, 606e) the hyperploid males again reveal the compound of scute-1 plus scute-8, and the single mutant character forked. The left-hand break therefore occurred between the loci of forked and carnation. All that can be said about the right-hand break in each of these cases, is that it must have occurred at some point lying between the locus of forked (inverted position) and the right or fiber-bearing end of the chromosome.

The results obtained in the last two cases (603c, 606q) give very definite evidence as to the position of the locus of scute-8. Here the hyperploid males showed scute-1 only, and the mutant character forked. Obviously, the locus of scute-8 had been eliminated in the deleted section. In each case the duplication fragment covers yellow, bobbed and carnation, and the left-hand break must have occurred between the loci of carnation and forked. If the locus of scute-8 lies next to that of yellow, it would be necessary to make the very highly improbable assumption that a double deletion had taken place; the first of these eliminating the locus of scute-8, the second eliminating a section lying between the locus of forked and the right end. A much simpler and more probable assumption is that the locus of scute-8 is inverted and lies near the fiber-bearing end. On this basis, the right-hand break took place between the locus of scute-8 and the right end. In the first eleven cases, then, the right-hand break must have occurred at some point lying to the left of the locus of scute-8.

It is possible to devise an experiment to test the validity of the conclusion that the locus of scute-8 does not lie at the left end of this X chromosome. This can be done by X-raying scute-8 males and mating them to yellow scute-1 females. The F_1 yellow aberrant or hypoploid female will show whether the locus of scute-8 has been eliminated along with that for yellow. If such a female reveals the character scute-1, then the locus of scute-8 must have been eliminated, and this would indicate that this locus must be situated close to that of yellow. If, however, the female shows the combined effect of scute-1 and scute-8, then the locus of scute-8 must not lie near the left end of the chromosome. This test was recently carried out by treating scute-8 apricot males and crossing them to yellow scute-1 vermilion carnation females. The F_1 cultures yielded sixteen yellow hypoploid females, and it was found that without exception these females showed the compound effect of scute-1 and scute-8. These results are listed in table 2.

TABLE 2

Shows effects of scute-1 and scute-8 and their compound.

TYPES OF FLIES	FIRST ORBITAL	SECOND ORBITAL	OCELLAR	POST-VERTICAL	ANTERIOR SCUTELAR	POSTERIOR SCUTELAR	FIRST NOTOPLEURAL
Scute-1	0.00	0.52	0.15	0.01	0.46	0.27	0.14
Scute-8	0.98	0.72	1.00	1.00	0.78	0.77	1.00
Scute-1+8 compound	0.89	0.90	1.00	0.92	0.58	0.45	0.98
Yellow hypoploid	0.93	0.84	1.00	0.84	0.43	0.56	0.75
621·a	2	2	2	2	1	2	0
621·b	2	2	2	2	2	2	2
621·d	1	1	2	1	0	0	0
621·h	2	2	2	2	0	0	1
621·k	2	1	2	2	2	1	2
622·a	2	0	2	0	0	1	2
622·c	2	2	2	1	1	2	2
622·f	2	2	2	2	1	2	2
622·g	2	2	2	2	2	2	1
622·j	2	2	2	2	1	2	2
622·k	1	1	2	2	0	0	0
622·m	2	2	2	1	0	0	2
622·t	2	2	2	2	0	0	2
622·v	2	2	2	2	2	2	2
622·w	2	2	2	2	1	1	2
622·z	2	2	2	2	1	1	2

It is desirable to explain the tabulated data presented in this table. The scute allele of the parent females, designated as scute-1, was found to affect the following major bristles located in the upper parts of the head and thorax: the first and second orbitals, ocellars, postverticals, anterior and posterior scutelars, and first notopleurals. The figure given

for each of these seven bristles, in the first (horizontal) line of the table, represents the average number of bristles present on one side of the fly. This was determined by examining one hundred parent females and recording the presence or absence of any of these bristles. From the data thus obtained, the average numbers are easily derived. These numbers really represent the percentage of presence for the several bristles. The figures show that in scute-1 females the first orbital is always absent, while the remaining six bristles are present to a variable extent.

The number for the same set of bristles for scute-8 were obtained in the same manner, by studying one hundred scute-8 males. An examination of these numbers, as given in the second line of the table, shows that scute-8 does not affect the ocellars, postverticals, or first notopleurals, for these are one hundred percent present. It only slightly affects the first orbitals, and has less effect than scute-1 on the second orbitals, the anterior and posterior scutelars.

When a scute-1 female is crossed to a scute-8 male the F_1 females show the combined effects of these two alleles. This phenotypic expression has been called the "compound" by the Russian workers. In the case of the present experiment, the percentages were determined from data obtained in examining one hundred F_1 non-variant females. These figures are shown in the third line of the table, and from the numbers there given, it can be seen that the F_1 compound female can easily be distinguished from the scute-1 female.

There is, however, considerable variation in the phenotypic expression of the two scute alleles in the compound. Some of the F_1 non-variant females show no bristles missing, while others may have several bristles absent. The most consistent effect is on the first orbitals, the ocellars, the postverticals, and the first notopleurals. These four pairs are almost always absent in scute-1 flies, but are usually present in the compound. They therefore constitute the best criteria by which to judge whether a given yellow hypoploid female represents a compound or only a scute-1 fly.

The average for the seven pairs of bristles of the yellow hypoploid females obtained in the experiment are given in the fourth line of the table. These averages compare very favorably with those for the corresponding ones for their gray non-variant sisters, especially in view of the fact that they had to be computed from only sixteen instead of one hundred flies. The individual records of these sixteen yellow females are listed in the fifth to twentieth lines of the table. The records show that without exception these yellow hypoploid females reveal the compound of scute-1 plus scute-8.

Genetic tests carried out on the yellow hypoploid females further support the conclusion reached above. These females were crossed to

scute-1 forked carnation bobbed males. Fourteen of them proved to be fertile, and gave results which showed that one of the fourteen was deficient at the locus of bobbed (inverted position) as well as at the locus of yellow. Hence, the locus of scute-8 cannot lie between the loci of yellow and bobbed.

The cultures yielding the sixteen yellow hypoploid females also gave fifteen hyperploid males, each carrying a duplication produced by a long deletion in the treated scute-8 chromosome. These males were all gray vermilion carnation flies, but with respect to the effect of scute, they belonged to two classes. Eleven of them showed scute-1 only, while the other four were compounds of scute-1 plus scute-8. We know from the results given in table 1, that the order of the genes in the scute-8 chromosome is, from left to right, yellow, bobbed, carnation, forked, etc.; hence, since the deletion producing the type of these four males eliminated the loci of both carnation and vermilion, and not that of scute-8, the latter must lie some place to the right of the locus of vermilion. Unpublished crossover data show that the locus of scute is in its normal position with reference to apricot, echinus and vermilion. We conclude, therefore, that the scute-8 locus lies near the spindle-fiber end of the chromosome.

THE POSITION OF THE ACHAETE LOCUS

The test for the locus of achaete was made possible by the use of an eversporting gray-yellow mosaic stock of scute-8, obtained in 1932 (PATTERSON 1933, p. 44). In view of the fact that this stock (carried balanced with yellow white) never gives scute-8 apricot males, spotting must be due to a spontaneous elimination of the left or gray end of the chromosome during somatogenesis.

Proof of this is shown by the results obtained in the following experiment. If scute-8 apricot females, heterozygous for yellow white are crossed to yellow white crossveinless forked-5 males, the yellow areas of the F_1 spotted females show none of the other recessive genes for which they are heterozygous. However, when these yellow spotted females are mated to yellow achaete males, the yellow spots of their daughters invariably show the character achaete, whenever such spots fall upon an area affected by this gene. This demonstrates that the normal allele of achaete lies next to that of yellow, and is consequently eliminated along with that of yellow when the end breaks off.

We conclude from these observations that the breaks which produced the long inversion found in the scute-8 chromosome, occurred between achaete and scute on the left and between bobbed and the spindle-fiber end on the right, the entire section between these points being inverted.

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CROSSING OVER NEAR THE SPINDLE ATTACHMENT OF THE X CHROMOSOMES IN ATTACHED-X TRIPLOIDS OF DROSOPHILA MELANOGASTER

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Received May 14, 1934

THE PROBLEM

A study of crossing over in triploid females of *Drosophila melanogaster* (BEADLE 1934) with two of the X chromosomes attached showed that crossing over near the spindle attachment is higher between the two attached chromosomes than between either of the attached chromosomes and the free chromosome. In more distal regions (beyond forked) the chromosomes were found to be involved in crossovers at random. Since these facts have a bearing on the mode of prophase pairing of chromosomes, it was thought worth while to check the experiment insofar as it concerned crossing over in the proximal portions of the chromosomes. Since only a relatively small fraction of the genetic length of the X chromosome was to be studied, the experiment could be much simplified over the original. Because of the less complicated genetic set-up in the X chromosomes it was possible to plan the experiment so that it would yield information on any relation which might exist between autosomal disjunction and detected crossing over in the X chromosome. That such a relation might be important was suggested by results obtained by Doctors J. SCHULTZ and H. REDFIELD of this laboratory (unpublished) in studies of free X chromosome triploids.

The results confirm the finding of the previous experiment that crossing over near the spindle attachment apparently does not involve chromosomes at random, but they go farther in indicating that the interpretation of this fact must take into account a relation shown to exist between recovered crossovers in the X chromosome and the type of disjunction of the autosomes.

PLAN OF THE EXPERIMENT

In planning an experiment which would give the desired information, several considerations were of importance. It was desirable to carry out the experiment in such a way that the phenotypic frequencies of the progeny of triploids of the proper constitution could be directly used as a measure of crossing over in the several types of gametes to be studied. The alternative, testing for genotypic constitution of individuals getting more than one X chromosome strand from their mothers, must be resorted

to if any considerable portion of the X chromosome is to be followed but has the serious disadvantage that its use is excluded in the case of intersexes, which are sterile. Intersex classes are relatively weak and their viability is therefore strongly influenced by many of the characters ordinarily used in genetic studies. Since it was highly desirable to make use of intersexes, the minimum possible number of genes consistent with efficiency was used and these were selected after due consideration had been given to their effects on viability. The use of the *cIIIG* gene was avoided as a means of obtaining triploids of the proper constitution since it was known from previous experience (BEADLE 1934) that a high proportion of the triploid daughters of $\widehat{XXY}$ *cIIIG* females are $\widehat{XXXY}$ in constitution; the Y chromosome is an undesirable complication.

Triploids of the desired constitution were built up by first making up a stock of $\widehat{XXX}$ triploids of the constitution *v f cr/v f B/v f cr*. This stock could be maintained and multiplied with little danger of change due to

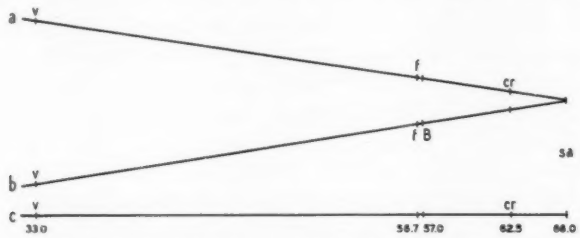


FIGURE 1.—Genetic constitution of the triploids used for a study of crossing over. The three chromosomes are designated a, b, and c. Standard map positions of the genes used as markers are indicated. The spindle attachment end is indicated by "sa" at the right.

crossing over. The only single crossover which could give a phenotypically undetectable change in constitution is a reciprocal between the attached chromosomes in the forked (*f*)-carnation (*cr*) interval. Such a change would not decrease the usefulness of the stock and the fact of its having occurred could be detected in the next generation. Such females were mated to vermilion (*v*) carnation males. Vermilion forked Bar ($B/+/+$ which can be distinguished from $B/B/+$) triploids were selected from this mating. They were of the proper constitution for the experiment (figure 1). Such females were mated to forked carnation males and allowed to lay eggs for a seven day period after which they were discarded. At the end of this seven day period the cultures were removed from the 25° C incubator to room temperature (about 20°) and allowed to remain until the counts were completed. The progeny were classified at one or two day intervals for eleven days.

The triploid females used in the experiment were homozygous for the vermilion gene, the males were not-vermilion; hence any individual in the progeny with two X chromosomes (females and intersexes) which showed vermilion got both of these chromosomes from the mother. Thus the female and intersex progeny could be classified phenotypically as having either attached or free X chromosomes.

The genetic constitution of the triploids is such that crossing over between the spindle attachment and forked (or Bar since *f-B* crossing over is negligible in frequency) can be detected in any of the three possible combinations of two chromosomes. In certain cases the interval under control can be separated into two, spindle attachment-carnation (region 1) and carnation-forked (region 2). Unless otherwise stated, crossover values in the discussion of the data refer to the total of the two regions, that is, spindle attachment-forked.

PROGENY OF $\widehat{XXX}$ TRIPLOIDS

A summary of the progeny of 123 triploid females is given in table 1. These data are more useful in indicating the types of individuals produced by $\widehat{XXX}$ triploids than are those from the previous experiment already referred to both because the numbers are larger and because the genetic set-up is such that more information is available.

TABLE 1

Summary of the progeny of 123 $\widehat{XXX}$ females.

CLASS	NUMBER
Regular males	996
Patroclinous males	4
XX females	1186
$\widehat{XX}$ females	1968
3n females	871
Matroclinous 3n females	1
XX intersexes	1854
$\widehat{XX}$ intersexes	700
Superfemales	55
Matroclinous superfemales	3
Supermales	182
Patroclinous supermales	13
Total	7833

It is well known that the distribution of chromosomes at meiosis in the free-X triploid of Drosophila is not random but deviates in such a way that the tendency is for equal numbers of chromosomes to be distributed to the two poles at the first division. This is shown clearly by the data published by BRIDGES and ANDERSON (1925) and which are given in table 2

of this paper. The same deviation from a random distribution is shown in $\widehat{XXX}$ triploids but it is much less extreme (table 2). The proportion of triploid females and diploid males from $\widehat{XXX}$ triploids is higher than from XXX triploids (BEADLE, l.c.) and is, of course, an expression of the difference in the deviation from random distribution of chromosomes in the

TABLE 2

Comparison of frequencies of different types of gametes from XXX and $\widehat{XXX}$ triploids.

EGG	SPERM	ZYGOTE	FREQUENCY		RELATIVE FREQUENCY	
			XXX*	$\widehat{XXX}$	XXX	$\widehat{XXX}$
2X, 1A	YA	2n female	1116	1968	7.2	1.7
1X, 1A	XA	2n female	154	1186	1.0	1.0
2X, 2A	YA	Intersex	104	700	1.0	1.0
1X, 2A	XA	Intersex	898	1854	8.9	2.7

* Data from BRIDGES and ANDERSON (1925).

two types of triploids. The cause of the deviation is unknown but the difference between $\widehat{XXX}$ and XXX triploids shows that both the mass of chromatin and the number of independent units (chromosomes) influence the distribution.

The summary given in table 1 shows that $\widehat{XXX}$ triploids produce several types in addition to those regularly found in the progeny of triploids. These are: matroclinous (for the X) triploids and matroclinous superfemales (from $\widehat{XXX}$, 2A and $\widehat{XXX}$, 1A eggs), and patroclinous males and patroclinous supermales (from noX, 1A and noX, 2A eggs). The experiment with $\widehat{XXX}$ triploids referred to above showed that patroclinous males are rather frequent among the progeny of $\widehat{XXX}$Y triploids (13.7 percent of all males). With this fact in mind, tests were made for fertility of the patroclinous males in this experiment. Of 3 tested 2 were sterile and 1 was fertile. There is evidence therefore that at least one of the 123 triploid parents carried a Y chromosome. Probably few or no others were of this constitution, otherwise there should have been an appreciable number of patroclinous males. It is interesting to compare the relative frequencies of $\widehat{XXX}$, 2A and $\widehat{XXX}$, 1A and those of noX, 2A and noX, 1A gametes. The first two types are recovered in matroclinous triploids and in matroclinous superfemales. The ratios of these to regular triploids and superfemales must be used to correct for the violent difference in viability of the two sex types. The relative frequencies, so corrected, are 1:48 for $\widehat{XXX}$, 2A and $\widehat{XXX}$, 1A gametes. NoX, 1A and noX, 2A

gametes are recovered in patroclinous males and in patroclinous supermales. The ratio of these two types (excluding the one culture the mother of which was shown to be $\widehat{XXXY}$), corrected for viability differences, is 1:24. These ratios are based on relatively small numbers but nevertheless indicate extreme deviations from the 1:1 relation expected with independent assortment of the chromosomes. They show that the frequency of large deviations from an approximately equal distribution of the chromosomes to the two poles of the first meiotic spindle is very much lower than that expected with random distribution.

CROSSING OVER

Since intersex classes are to be used in measuring crossing over in certain types of gametes produced by triploids, it is necessary to examine the data to see whether or not viability complications might invalidate comparisons between values based on normal and those based on intersex classes. The experiment is so arranged that crossovers recovered in $\widehat{XX}$, 2A gametes can be directly compared in the two sex classes, triploids and $\widehat{XX}$ intersexes. These two classes are differentiated by the sperm and should, aside from viability differences, give similar results. The frequencies of different crossover types in these classes are given in table 3. The percentages of detected crossovers between the chromosomes of the three combinations are summarized as follows:

CHROMOSOMES	TRIPLOIDS	INTERSEXES	DIFFERENCE	S.E.
a–b	3.04	3.57	0.53	0.82
b–c	7.01	7.29	0.28	1.31
a–c	7.23	7.30	0.07	1.33

The values for a–b crossovers represent only one half the frequency of potentially detectable crossovers. They are presented in this way because of uncertain classification of one type of crossover (*v f BB*) in the $\widehat{XX}$ intersexes. It can be seen that the values from the two sex classes agree very well; all of the differences are considerably less than their standard errors. This agreement means that there are no appreciable differential inviability effects in the different phenotypes used as measures of crossover frequencies, and it is therefore legitimate, in this experiment, to compare crossover values based on intersex classes with those based on normal sex classes.

Crossover Values in Different Types of Gametes

There are three combinations of two chromosomes in which crossovers can be detected in the experiment as set up (a–b, a–c, and b–c in figure 1).

The relative frequencies of these in different classes of individuals are given in table 3. Crossovers between the two attached chromosomes

TABLE 3

Progeny of $\widehat{XXX}$ trilpoids (v f cr/v f B/v cr) mated to f cr males.

PROGENY CLASS	CHROMOSOMES	REGION	PHENOTYPE	NUMBER	PERCENT
Males	c	0	*v cr*	844	
(996)	c-b	1	*v f B*	45	4.51
		2	*v f B cr*	24	2.41
	c-a	1 or 2	*v f cr*	81	8.13
	c-b	1 and 2	*v*	2	0.20
XX females	c	0	*cr*	1026	
(1186)	c-b	1	*f B*	54	4.55
		2	*f B cr*	28	2.36
	c-a	1 or 2	*f cr*	75	6.32
	c-b	1 and 2	+	2	0.17
			f	1*	0.08
Males and XX females	c	0		1870	
(2182)	c-b	1		99	4.53
		2		52	2.38
	c-a	1 or 2		156	7.14
	c-b	1 and 2		4	0.18
	c-b-a	1 and 2		1*	0.05
XX intersexes	c	0	*cr*	1725	
(1854)	c-b	1	*f B*	32	1.73
		2	*f B cr*	32	1.73
	c-a	1 or 2	*f cr*	64	3.45
	c-b	1 and 2	+	1	0.05
Triploids		0	*f B*	694	
(871)	a-b	1	*f cr*	17	1.95
		2	*f*	7	0.80
		1 or 2	*f BB*	28	3.22
	b-c	1	*cr*	43	4.95
		2	+	18	2.06
	a-c	1 or 2	*B*	61	7.00
			B cr	2*	0.23
			BB	1†	0.11
$\widehat{XX}$ intersexes		0	*v f B*	566	
(700)	a-b	1	*v f cr*	15	2.14
		2	*v f*	10	1.43
		1 or 2	*v f BB*	7	
	b-c	1	*v cr*	33	4.72
		2	*v*	18	2.57
	a-c	1 or 2	*v B*	51	7.30

TABLE 3 (*Continued*)

PROGENY CLASS	CHROMOSOMES	REGION	PHENOTYPE	NUMBER	PERCENT
$\widehat{XX}$ females		0	*v f B*	1657	
(1968)	a-b	1	*v f cr*	53	2.70
		2	*v f*	23	1.17
		1 or 2	*v f BB*	67	3.40
	b-c	1	*v cr*	40	2.03
		2	*v*	32	1.65
	a-c	1 or 2	*v B*	92	4.68
			v f B cr	3‡	0.15
			v B cr	1*	0.05

* From $\widehat{XXX}$ parent of the constitution *v f/v f B cr/v cr*—should be added to c-a single crossovers.

† a-b-c- double crossover with the b-c crossover between *f* and *B*—treated as a-b single.

‡ b-a-b or b-c-b doubles in regions 1 and 2—because a-b crossing over is more frequent in this type of gamete and because b-a-b doubles are detected with twice the frequency of b-c-b doubles, these are treated as b-a-b crossover.

(a–b) will be considered first. The detected crossover frequencies (one half the total exchange frequency between the two designated chromosomes) for different types of gametes are summarized as follows:

GAMETE	SPERM	ZYGOTE	PERCENT CROSSOVERS	S.E.
$\widehat{XX}$, 2A	XA	3n female	6.08	0.85
	YA	$\widehat{XX}$ intersex	7.14	1.40
$\widehat{XX}$, 1A	YA	$\widehat{XX}$ female	7.43	0.59

The value for $\widehat{XX}$ intersexes, 7.16, is obtained by multiplying the homozygosis value for chromosome a by two; homozygosis for Bar (chromosome b) cannot be measured because of difficulties of classification for this gene in intersexes. The values are in fair agreement. The average of the two values for $\widehat{XX}$, 2A gametes differs from the value for $\widehat{XX}$, 1A gametes by 0.81 percent, a value less than its standard error. Thus there is no indication that crossing over between the two attached chromosomes is any different in the two types of gametes in which it can be measured.

The frequencies of detected crossovers between one of the attached chromosomes and the free chromosome (a-c or b-c) are:

GAMETE	SPERM	ZYGOTE	CHROMOSOMES	PERCENT CROSSOVERS	S.E.
1X, 1A	YA or XA	2n male or female	a-c	7.19	
			b-c	7.27	
			Total	14.36	0.75
1X, 2A	XA	XX intersex	a-c	3.45	
			b-c	3.56	
			Total	7.01	0.62
$\widehat{XX}$, 1A	YA	$\widehat{XX}$ female	a-c	4.73	
			b-c	3.68	
			Total	8.41	0.62
$\widehat{XX}$, 2A	XA	3n female	a-c	7.23	
			b-c	7.01	
			Total	14.24	1.19
	YA	$\widehat{XX}$ intersex	a-c	7.30	
			b-c	7.29	
			Total	14.59	1.35

In contrast to those for crossing over between the two attached chromosomes, it is clear that these values show striking differences depending on whether one or two sets of autosomes were present in the egg. The gamete types 1X, 1A and $\widehat{XX}$, 2A give values about twice the magnitude of the comparable values for 1X, 2A and $\widehat{XX}$, 1A gametes. The difference between 1X, 1A and 1X, 2A values is 7.6 times its standard error; that between $\widehat{XX}$, 1A, and $\widehat{XX}$, 2A is 5.5 times its standard error. There can be no question as to the statistical significance of these differences. It is clear, then, that crossing over between attached and free chromosomes in regions near the spindle attachment is in some way related to autosomal disjunction. An inspection of the separate values for regions 1 and 2 which are based on smaller numbers and therefore less reliable than the combined values, suggests that the differences are greater in region 1 (nearest the spindle attachment) than in region 2; the data are inadequate to establish clearly this relation.

Crossover values between different homologs

For comparison of crossover values between the two attached chromosomes with those between attached and free chromosomes the data can be summarized as follows:

GAMETE	SPERM	ZYGOTE	PERCENT CROSSOVERS A-B	PERCENT CROSSOVERS A-C OR B-C	DIFFERENCE	S.E.
$\widehat{XX}$, 1A	YA	$\widehat{XX}$ female	7.43	4.20	3.23	0.67
$\widehat{XX}$, 2A	XA	3n female	6.08	7.12	1.04	1.04
$\widehat{XX}$, 2A	YA	$\widehat{XX}$ intersex	7.14	7.30	0.16	1.56

These values are comparable and represent detected crossing over (one half total exchange) for one combination of two chromosomes; the "a-c or b-c" values are the average of the two. It can be seen that in $\widehat{XX}$, 1A gametes crossing over between the attached chromosomes (a-b) is significantly higher than between attached and free chromosomes (a-c or b-c); the difference is 4.8 times its standard error. It is a noteworthy fact that such a difference does not exist in $\widehat{XX}$, 2A gametes. In fact the observed differences are in the opposite direction but are clearly not statistically significant; in one case the difference is equal to its standard error, in the other it is much less.

Comparison of triploid and diploid exchange values

A comparison of crossing over near the spindle attachment of the X chromosome in triploids with that of diploids is complicated by the relation between crossing over in this region in the triploid and the type of disjunction of the autosomes. It is evident that the triploid values will vary

TABLE 4

Homozygosis values in XX daughters of triploids used in this experiment.

CONSTITUTION	PROGENY	CROSSOVER REGION	NUMBER	PERCENT
$\frac{vfcr}{vfB}$	*vfB*	0	3416	
	vfcr	1	47	1.30
	vf	2	63	1.74
	vfBB	1 or 2	94	2.60
$\frac{vfcr}{v\ cr}$	*vcr*	0	1138	
	vfcr	1 or 2	41	3.48

widely depending on the type of gamete in which they are measured. A question also arises as to the choice of a diploid control. Crossing over in diploid $\widehat{XX}$ daughters of the triploids used in the experiment has been measured. This was done in non-crossover $\widehat{XX}$ daughters and also in $\widehat{XX}$ daughters in which the b chromosome had been replaced, by crossing over in region 1, with the c chromosome. The data are given in table 4. The two values agree but both are lower than standard values (see BEADLE and EMERSON 1935, for discussion of this difference). The total exchange in the triploid calculated in various ways, together with the standard diploid value (twice standard map distance) and the value obtained in the $\widehat{XX}$ diploid control (twice or four times homozygosis values, depending on whether homozygosis for both or one chromosome could be detected) are:

	Percent Exchange
Standard diploid	18.6
$\widehat{XX}$ diploid	12.6
$\widehat{XX}$, 2A gametes in 3n daughters	40.6
$\widehat{XX}$, 2A gametes in intersexes	43.5
$\widehat{XX}$, 1A gametes in $\widehat{XX}$ daughters	31.7
$\widehat{XX}$, 2A and X, 2A gametes	27.2
Average of all gametes	35.9

It is clear that there is a marked regional increase near the spindle attachment in the triploid as compared with the diploid. These results corroborate those previously obtained (BEADLE 1934).

From the experiment it is possible to determine whether or not the two marked regions are increased proportionately in the triploid. This can conveniently be done by comparing the ratios of crossover values for the two regions in the triploid and diploid. The ratios (regions 1:2) are:

Standard	1:1.7
$\widehat{XX}$ diploid	1:1.3
Triploid	1:0.6

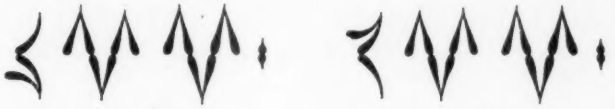
The ratio for the triploid represents the unweighted average of the ratios for the four types of gametes. In all types of gametes the triploid shows a higher proportion of crossovers in region 1 than does the diploid. It is therefore quite clear that the increase in crossing over in the triploid falls off progressively with distance from the spindle attachment (the distal regions of the chromosome are of course not considered here).

DISCUSSION

Relation of autosomal disjunction and X chromosome crossing over

The evidence presented above demonstrates that there is a relation in attached-X triploids between disjunction of the autosomes and crossing over near the spindle attachment between attached- and free-X chromosomes. This relation is such that attached and free chromosomes show low crossing over in gametes which result from disjunction which gives as near equal numbers of chromosomes to the two poles of the first meiotic spindle as is consistent with the formation of gametes which can give viable zygotes with normal spermatozoa, and high crossing over in gametes from disjunction which distributes two members of each of the three major

groups of homologues to one pole and one member of each to the other pole of the spindle. These two types of disjunction are illustrated in figure 2. From the fact that the chromosomes of triploids are not distributed to the two poles of the first meiotic spindle at random it is known that there must be a correlation in the orientation of different potential trivalents on the equatorial plate. The evidence presented here shows that such selective orientation of the $\widehat{XXX}$ trivalent with respect to the autosome trivalents is influenced by crossing over near the spindle attachment, between attached and free chromosomes, in such a way that if crossovers occur in this region, the $\widehat{XXX}$ trivalent is less likely to be selectively oriented than if such crossovers are absent. A possible explanation of the facts can be made on the basis of two assumptions, (1) a general and non-specific force



A B

FIGURE 2.—Representation of the two types of disjunction differentiated in the present study. A—1X, 2A:XX, 1A type which gives low crossing over between attached and free chromosomes. B—XX, 2A: 1X, 1A type which gives high crossing over between attached and free chromosomes.

of repulsion acting at and before metaphase (LILLIE 1905, KUWADA 1929, DARLINGTON 1932) and (2) a relation between exchanges and metaphase association of homologous chromosomes (DARLINGTON 1931). If exchanges are assumed to hold homologs together at the point of crossing over during their orientation on the equatorial plate, then, following an exchange near the spindle attachment between one attached and the free-X chromosome, the members of the $\widehat{XXX}$ trivalent will be so associated that they will bear approximately the same relation to the general mass of autosomes regardless of their orientation. If, on the other hand, the free-X is distally associated with one attached chromosome, a change in orientation of the $\widehat{XXX}$ trivalent will considerably change the relation of its mass to that of the autosomes; hence such a type of $\widehat{XXX}$ trivalent can easily be imagined to be selectively oriented with respect to the autosomes. In a similar way, the difference between X chromosome disjunction in triploids with free-X's and those with two of the X's attached can be accounted for.

It is evident that the relation discussed above has a bearing on the problem of measuring crossing over in spindle attachment regions in triploids. The reciprocal relation to that shown here, namely, that between auto-

somal crossing over and X chromosome disjunction has not been investigated but should be taken into account in any attempt to measure accurately crossing over in spindle attachment regions.

Prophase pairing of chromosomes

The fact that in $\widehat{XX}$, 1A gametes the two attached chromosomes cross over more frequently with each other than does either with the free chromosome was previously interpreted (BEADLE 1934) as indicating that prophase pairing is by twos and that the two attached chromosomes have a pairing advantage. The fact that this relation does not hold in all types of gametes necessitates a reconsideration of the interpretation. Considering all types of gametes the net difference in crossing over is in favor of the attached chromosomes, that is, the average of the high and low values for attached and free chromosome crossing over is less than the average value for crossing over between the two attached chromosomes. These values are:

a-b	6.9
a-c or b-c	5.5
Difference	1.4
Standard error	0.6

The difference is not large but is probably statistically significant. However the evidence for the interpretation previously given is considerably weakened by the additional information now available and the question as to the nature of prophase pairing in triploids of Drosophila must, as far as evidence in this organism goes, remain open.

SUMMARY

Crossing over was studied near the spindle attachment of the X chromosomes in triploids of *Drosophila melanogaster* with two attached chromosomes. The experiment was so arranged that crossovers could be detected in four types of gametes, namely, 1X, 1A; 1X, 2A; $\widehat{XX}$, 1A; and $\widehat{XX}$, 2A.

The results suggest that crossing over between the two attached chromosomes is constant in the two types of gametes in which it can be studied. On the other hand, crossing over between either of the attached chromosomes and the free chromosome is markedly different in different types of gametes. It is low in those gametes which have as near half the total number of major chromosomes as is possible (1X, 2A and $\widehat{XX}$, 1A) and is high in those whose chromosome numbers are lower or higher than this (1X, 1A and $\widehat{XX}$, 2A).

Non-random distribution of chromosomes at meiosis in triploids of Drosophila must mean that there is a correlated orientation of chromosomes on the equatorial plate. The evidence summarized above shows that this selective orientation is influenced by crossing over.

The relation of the results to the problem of measuring crossing over in triploids and to the question of the nature of prophase pairing of chromosomes is discussed.

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FURTHER STUDIES OF CROSSING OVER IN ATTACHED-X CHROMOSOMES OF DROSOPHILA MELANOGASTER

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Received May 17, 1934

THE PROBLEM

For some years it has been known from genetic data that crossing over in the female of *Drosophila melanogaster* occurs at a stage of meiosis after the chromosomes have split equationally (BRIDGES 1916, L. V. MORGAN 1925, ANDERSON 1925, BRIDGES and ANDERSON 1925). More recently crossing over in several other organisms has been demonstrated, by genetic methods, to occur at such a stage of meiosis (RHOADES 1932—Zea; WHITING and GILMORE 1932—Habrobracon; LINDEGREN 1933—Neurospora; ALLEN 1930—Sphaerocaros; and others). The genetic evidence has been confirmed cytologically in Zea (MCCLINTOCK 1931). The uniformity, in essentials, of the meiotic mechanism as revealed by cytological studies of a large number of widely different organisms, considered in connection with the above mentioned genetic information justifies the generalization that in all organisms in which crossing over occurs at meiosis, it takes place after the equational division of the chromosomes. With the demonstration of this fact concerning the stage at which crossing over occurs, several questions arose relating to the process. Certain of these have received satisfactory answers; others have awaited the accumulation of additional data. Of the latter, the relation of the strands involved in a given crossover to those involves in other crossovers has not, as yet, been settled with the degree of certainty desired. Many problems of crossing over are inseparably associated with this question. For example, an understanding of interference in crossing over as expressed in single recovered strands is dependent on the relation of strands involved in adjacent crossovers (HALDANE 1931, WEINSTEIN 1932). Likewise the randomness or non-randomness of strands involved in different crossovers bears directly on the relation of crossover to recombination frequencies (EMERSON and RHOADES 1933). Finally, any theoretical consideration of the mechanism involved in the process of crossing over must take into account the relation of the individual events involved in multiple crossing over (SAX 1932, BELLING 1933).

MATERIALS AND METHODS

The ideal organism for a study of the problem defined above is one in which the four products of a single meiosis can be investigated. This con-

dition is realized in certain plants in which the products of meiosis remain together until maturity, for example in the flowering plant Epilobium (MICHAELIS 1931), in mosses (WETTSTEIN 1924), in liverworts (ALLEN 1924) and in many of the fungi. The ascomycete Neurospora has the advantages of the plants mentioned above and the additional convenience that the products of the two meiotic divisions can be differentiated (LINDEGREN 1933). However, in none of these organisms has the genetic analysis been carried sufficiently far to yield results which bear on the question under consideration. In Drosophila the use of attached-X females provides a practicable means of getting at the problem. Here only two of the four strands of a given tetrad can be recovered but an analysis of these should be sufficient for the purpose outlined.

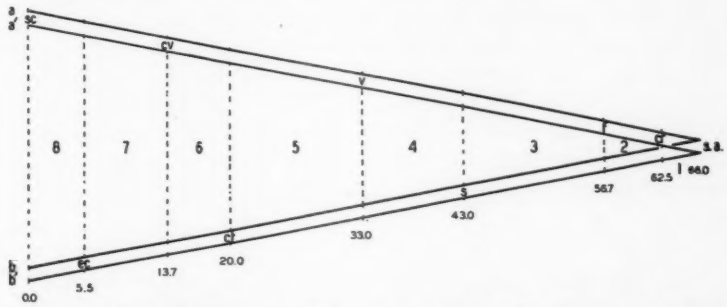

FIGURE 1.—Constitution of attached-X females used in the study reported in this paper. Crossover regions are indicated in larger numerals. Standard map positions are indicated by smaller numbers below. Spindle attachment is indicated by the letters "sa."

An attached-X stock of the desired constitution was built up from triploids with two attached chromosomes. By this method genes can be introduced into the attached chromosomes by crossing over. The stock obtained in this way had the genes scute (*sc*), crossveinless (*cv*), vermilion (*v*), forked (*f*), and carnation (*cr*) in one chromosome and echinus (*ec*), cut-6 (*ct*), and sable-2 (*s*) in the attached homolog. This constitution together with the standard map positions of the genes is shown in figure 1. The alternated position of the genes has the advantage of reducing viability complications. In such an experiment viability differences are of relatively great importance and to reduce them still further the number of offspring per culture bottle was kept low. Daughters of females of the proper constitution were mated to Bar males (for the purpose of identifying detachments) and allowed to lay eggs for a 3 day period, transferred to a second bottle and allowed to lay for a 2 day interval, then discarded. Since attached-X females produce fewer offspring than free-X females

(YY individuals lethal and XXX superfemales practically so), this procedure resulted in cultures with near the optimum number of individuals. A temperature of 25°C and a fairly humid atmosphere were maintained. On hatching, the progeny were classified; those from parents of other than the desired constitution (because of crossing over) were discarded. Wild-type females only (about 50 percent) were of course set up as parents. Of these approximately 56 percent were of the proper constitution.

For the purposes of the study it was necessary to determine the genotypic constitution of a considerable number of the offspring of females of the constitution given above. Certain cultures were selected and all of the females hatching in these were mated for constitution tests. In all, tests were obtained on 1478 females. Since the genotype of a given female is deduced from the types of equationals appearing among her daughters and since the frequency of equationals for a given gene is a function of the distance of that gene from the spindle attachment, the position of genes in the proximal region of the chromosome in relation to those more distally located is often difficult. It was found to be impossible to obtain tests for carnation in all of the females. Tests for forked were not always obtained in single cultures. Since it was highly desirable to have tests for forked for all of the females, those from which forked daughters were not

TABLE 1

Phenotypic frequencies among daughters of attached-X mothers of the constitution $\frac{sc\ cv\ v\ f\ cr}{ec\ ct\ s}$.

PHENOTYPE	FREQUENCY	PERCENT	S.E.	PHENOTYPE	FREQUENCY	PERCENT	S.E.
wild-type	4093	51.07	0.56	*v*	166	2.07	0.16
sc cv v f cr.	26	0.32	0.06	*f cr*	12	0.15	0.04
sc cv v f	47	0.59	0.09	*f*	18	0.22	0.05
sc cv v	456	5.69	0.26	*ct s*	118	1.47	0.14
sc cv	654	8.16	0.31	*ct*	93	1.16	0.12
sc	432	5.39	0.25	*s*	102	1.27	0.13
ec ct s	330	4.12	0.23	*sc cv f*	1	0.01	0.01
ec ct	645	8.06	0.31	*sc f*	2	0.02	0.02
ec	496	6.20	0.27	*sc f cr*	3	0.04	0.02
cv v f cr	11	0.14	0.04	*sc s*	5	0.06	0.03
cv v f	29	0.36	0.07	*ec f cr*	2	0.02	0.02
cv v	160	2.00	0.16	*ec v*	2	0.02	0.02
cv	58	0.72	0.10	*ec v f*	1	0.01	0.01
v f cr	9	0.11	0.04	*ec s*	3	0.04	0.02
v f	40	0.50	0.08				
				Total	8014		

obtained were tested further by raising mass cultures of the daughters. In this way definite tests were obtained for practically all of the females. Although there is a possibility of error in using tests obtained in the second generation, the magnitude of this error for the gene forked can be shown to be negligibly small. Females whose second generation tests showed no forked flies were tested in a third mass culture generation. The absence of forked in this generation was considered as definite evidence that the original tested female was homozygous for the normal allele of forked.

TABLE 2

Genotypic constitutions of all tested females with carnation disregarded. Single letter symbols are used for brevity. Equivalents are: scute, u; echinus, e; crossveinless, c; cut, t; vermilion, v; sable, s; forked, f. Phenotypes are indicated in bold face type followed by the total number of the given phenotype tested. Non-italicized symbols indicate that the genotype might equally probably be other than that given, for example, ucvf/ecv can be either ucvf/ecv or ucv/ecvf.

Wild-type (705)		**ucv (54)**		*uetvf/ets*	3	*ucvf/evf*	8	*ulf/ets*	5
		ucvf/ucv	31	*etvf/uets*	2	*uetvf/cvf*	1	*etf/uts*	1
ucvf/ets	398	*ucvf/ucvs*	22	*etv/ets*	1	*uevf/cvf*	1	*uctvf/ets*	3
etsf/ucv	39	*ucvsf/ucv*	1	**e (109)**		**v(45)**		*etvf/ucts*	1
etf/ucvs	46	**uc (90)**		*evf/ets*	38	*ucvf/etv*	4	*ulvf/ets*	2
etvf/ucs	82	*ucvf/ucs*	51	*ecvf/ets*	52	*etvf/ucv*	3	*etvf/uts*	2
evf/ucts	29	*ucvf/ucts*	33	*etsf/ev*	1	*ucvf/etv*	7	*ulf/etvs*	1
ecvf/uts	38	*ucsf/ucv*	2	*etsf/ecv*	5	*ucvf/ev*	3	**s (28)**	
cvf/ets	18	*uctsf/ucv*	2	*etf/evs*	3	*evf/ucv*	7	*ucvs/ets*	4
cvf/uets	18	*uctf/ucvs*	1	*etf/ecvs*	3	*ucvf/ev*	5	*ucs/ets*	6
ucv/ets	2	*uctvf/ucs*	1	*etvf/ecs*	1	*ucvf/etvs*	3	*ucvsf/ets*	1
ucvsf/et	1	**u (94)**		*uevf/ets*	2	*etvf/ucvs*	1	*etsf/ucvs*	1
ucsf/etv	1	*ucvf/uts*	42	*evf/uets*	3	*ucvf/evs*	7	*ucvsf/ets*	1
uctsf/ev	1	*ucvf/uets*	34	*ecv/ets*	1	*evf/ucvs*	3	*ucsf/ets*	2
utsf/ecv	2	*utsf/ucv*	4	**cvf (19)**		*uctvf/evs*	1	*etsf/ucs*	8
uetsf/cv	2	*uetsf/ucv*	2	*ucvf/ecvf*	12	*cvf/etvs*	1	*ucsf/ets*	4
etsf/cv	1	*ulf/ucvs*	3	*ucvf/cvf*	7	**f (22)**		*ucts/es*	1
ucf/ets	2	*uetf/ucvs*	2	**cv (27)**		*ucvf/etsf*	8	**uf (3)**	
etf/ucs	1	*utvf/ucs*	2	*ucvf/ecv*	5	*ucvf/etf*	10	*ucvf/utsf*	1
ucf/etvs	1	*uetvf/ucs*	4	*ucvf/ecv*	2	*etvf/ucsf*	1	*ucvf/uetf*	1
uctf/evs	2	*uevf/ucts*	1	*ucvf/cv*	1	*evf/uctsf*	1	*ucf/uetvf*	1
uetf/cvs	1	**ets (48)**		*cvf/ucv*	3	*ecvf/utsf*	1	**us (2)**	
etf/cvs	1	*ets/ets*	15	*ucvf/cv*	4	*cvf/uetf*	1	*uets/ucs*	1
ucvf/es	5	*etsf/ets*	27	*ucvf/ecvs*	5	**ts (22)**		*ucsf/uts*	1
uctvf/es	2	*uetsf/ets*	1	*ecvf/ucvs*		*ucts/ets*	4	**ef (1)**	
utvf/ecs	2	*uetsf/ets*	5	*ucvf/cvs*	3	*uts/ets*	1	*ecvf/etf*	1
uetvf/cs	3	**et (123)**		*cvf/ucvs*	1	*uctsf/ets*	1	**ev (1)**	
etvf/cs	3	*etf/ets*	37	**c (10)**		*etsf/ucts*	2	*ecvf/etv*	1
ucvf/ts	1	*etvf/ets*	69	*ucvf/ecs*	2	*ulsf/ets*	1	**evf (1)**	
cvf/uts	1	*etsf/et*	1	*ucvf/cs*	5	*etsf/uts*	2	*ecvf/etvf*	1
ecv/uts	1	*etsf/etv*	4	*cvf/ucs*	2	*utsf/ets*	11	TOTAL	1478
tsf/ecv	1	*etf/etvs*	1	*ucvf/ets*	1	**t (20)**			
ucvf (17)		*uetf/ets*	3	**vf (37)**		*uctf/ets*	2		
ucvf/ucvf	17	*etf/uets*	2	*ucvf/etvf*	27	*etf/ucts*	3		

EXPERIMENTAL DATA

Phenotypic types and their frequencies among daughters of females of the correct constitution (figure 1) are given in table 1.

The results of constitution tests are presented in tables 2 and 3. Because of the large numbers of classes the constitutions are given in abbreviated form as explained in the heading of the table. Partly because of differences

TABLE 3

Genotypic constitutions of females for which carnation tests were obtained. Only those showing crossovers in region 1 or 2 are included here since other regions can be treated from the data in table 2. These data are all included in table 2 with carnation disregarded. The symbols are the same as in table 2 with the addition of r for carnation. Phenotypes are given in bold face type followed by the total number of females of the given phenotype for which carnation tests were obtained. Non-italicized symbols are used as in table 2.

Wild-type (505)		**ets (19)**		*ucvft/ecvf*	3	**v (25)**		**ts (12)**	
etsr/ets	4	*etsr/ets*	4	*ucvfr/cvf*	1	*evsr/ucvf*	1	*uctsr/ets*	1
etsr/ucvf	7	**et (53)**		*ucvft/evf*	2	*ucvsr/evf*	1	**s (15)**	
ucvr/ets	1	*etsr/etvf*	2	**vfr (5)**		**fr (9)**		*ucvsr/ets*	1
etsr/cvf	1	*etsr/uetvf*	1	*ucvfr/etvfr*	4	*ucvfr/etsfr*	4	*etsr/ucs*	1
esr/ucvf	1	**e (71)**		*uetvfr/cvfr*	1	*ucvfr/utfr*	4	*u sr/ets*	2
ucvfr (7)		*etsr/ecvf*	2	**vf (7)**		*ecvfr/utsfr*	1	*uctsr/es*	1
ucvfr/ucvfr	7	*ecvr/etsf*	1	*ucvfr/etvf*	3	**f (4)**		**ufr (1)**	
ucvf (6)		**cvfr (6)**		*ucvft/etvf*	2	*etsfr/ucvf*	1	*ucvfr/uetfr*	1
ucvfr/ucvf	6	*ucvfr/ecvfr*	6	*ucvfr/evf*	1	*etvfr/ucsf*	1	**efr (1)**	
u (63)		**cvf (8)**		*evfr/ucvf*	1	*ucvfr/etf*	1	*ecvfr/etfr*	1
ucsr/uetvf	2	*ecvfr/ucvf*	2			*etfr/ucvf*	1		

in fertility and partly intentionally the different phenotypes were not tested in numbers exactly proportional to those in which they occurred. For this reason, in certain of the analyses, proportional frequencies must be used. These are obtained by multiplying the frequencies of the genotypes of a given phenotype by the frequency with which that phenotype occurred in the total of the counts of phenotypic constitution.

RIGHTMOST DETECTED CROSSOVERS

ANDERSON'S studies on crossing over in attached-X females of Drosophila (1925) indicated that the rightmost crossovers (those nearest the spindle attachment) are equally likely to involve two attached strands (reciprocal) or two strands, one from each pair of attached strands (equational)—(see EMERSON and BEADLE 1933 for diagrams). This is the expectation if the rightmost crossover involves any two non-sister strands at random. EMERSON and BEADLE (1933) summarized the data of ANDERSON (1925) and of STURTEVANT (1931) together with new data for the regions from vermilion (33.0) to Bar (56.7). All of the data are in substantial agreement. The results from the present study are given for in-

dividual regions in table 4. The three types aa/ab, ba/bb, and ba/ab are, assuming random crossing over between non-sister strands, expected in equal frequencies. The observed values agree very well with those expected. The totals of the proportional percentages for all regions are 24.56, 21.90 and 24.77 for the types as listed. These data therefore confirm the conclusions arrived at from previous data, namely that the crossover nearest the spindle attachment involves non-sister strands at random.

RELATION OF STRANDS INVOLVED IN DOUBLE CROSSOVERS

Excluding crossovers between sister strands, there are three types of double exchanges possible. These are designated two strands, three strand, and four strand. These types can be described by designating the strands a, a′, b, and b′. Two strand double exchanges involve the

TABLE 4

Proportional frequencies of different types of single crossovers. The numbers in italics represent the rightmost crossovers in crossovers of higher rank.

CROSSOVER REGION	aa/ab	ba/bb	ba/ab
1	0.32		
	0.49		
x*	0.91	1.29	
	1.63	*1.18*	
2	0.59	0.87	0.71
	1.10	*0.63*	*1.25*
3	3.26	2.32	2.82
	2.47	*2.46*	*2.11*
4	2.32	2.42	3.33
	1.63	*1.18*	*1.19*
5	4.63	4.52	5.94
	1.00	*0.80*	*1.21*
6	2.99	2.16	2.10
	0.07	*0.28*	*0.06*
7	2.41	2.96	2.75
	0.14		
8	1.95	1.30	1.30
Total (2 to 8)	24.56	21.90	24.77
Calculated	23.74	23.74	23.74

* Region x is used for regions 1 and 2 combined in cases where they cannot be differentiated.

same two strands at the two levels, for example, a and b at level 1 and a and b at level 2. Three strand doubles involve three different strands at the two levels, for example, a and b at level 1 and a and b′ or a′ and b at level 2. Four strand doubles involve two strands at one level and the two remaining strands at the second level, for example, a and b at level 1 and a′ and b′ at level 2. With random crossing over between non-sister strands, these types are expected to occur in the ratio 1:2:1.

From the two strands recovered in the daughters of attached-X females certain genotypes can be used as a measure of the frequency of different types of double exchanges. First, consider the frequencies of two and four strand doubles. The type aaa/bab or aba/bbb can come from either two or three strand doubles. The type baa/aab or baa/abb results from either

TABLE 5

Frequencies of genotypes which measure the relative frequencies of two and four strand double exchanges.

CROSSOVER REGIONS	aaa / bab	baa / aab	aba / bbb	bba / abb
2–3	1	1	1	0
2–4	1	1	0	0
2–5	3	0	0	1
2–6	1	1	0	0
2–7	0	2	0	0
2–8	1	0	0	0
3–4	0	0	1	1
3–5	4	3	2	8
3–6	3	7	1	2
3–7	5	0	1	2
3–8	1	3	1	0
4–5	4	2	2	1
4–6	7	3	2	3
4–7	5	3	5	1
4–8	3	1	0	2
5–6	5	0	3	1
5–7	2	0	2	2
5–8	5	2	3	2
6–8	1	0	2	3
7–8	1	1	0	0
Totals	53	30	26	29

three or four strand double exchanges. Since three strand doubles contribute equally to the two classes (complementary products of one double exchange) it follows that if the two types are equal in frequency, two and four strand double exchanges occur equally often (see EMERSON and BEADLE 1933, for diagrams.) EMERSON and BEADLE (1933) give a summary of the available data which show that the two types occur with frequencies which show no statistically significant difference. The

relative frequencies of these types for different pairs of regions are given in table 5. The totals in absolute numbers are 53:30 for aaa/bab and baa/aab; 26:29 for aba/bbb and bba/abb. The deviation from a 1:1 ratio in the first comparison is about 3.8 times its probable error. The second difference is in the opposite direction but is not significant The sum of the two pairs, 79:59, shows a deviation from a 1:1 ratio which is about 2.5 times its probable error. These data combined with the totals (corrected) given by EMERSON and BEADLE (1933) give totals of 152:121 or percentages of 55.6 to 44.4. The deviation from equality is 2.8 times its probable error. There is an excess of two over four strand types but it is of doubtful statistical significance. In such a determination as the above there are certain sources of error which should be considered in evaluating the observations. Viability differences should not exist since the types compared are of identical phenotypes. Mutation is a possible source of error that should be taken into account. Lethal mutations (at single loci or associated with chromosome aberrations) would eliminate individuals homozygous for the lethal gene and would give apparent two strand doubles in regions adjoining a marker gene close to the lethal. Numerous published control experiments in induced mutation studies show that about 2.5 lethal mutations per thousand X chromosomes can be expected per generation. Thus among the total of the females tested in this and previous studies (3308), 17 lethal mutations might reasonably be assumed to have been present. Granting the legitimacy of such a correction, the deviation from equality of two and four strand types would be reduced to a value not much larger than its probable error. Unfortunately in most of the tests, counts were not made so there is no way of directly checking possible errors due to mutation. However we can gain some idea of the reasonableness of the assumption that a few such errors do exist by considering the ratio of the two types in doubles in adjacent regions. Doing this for the present study (table 5), we find a ratio of 18:7. If the excess of two strand doubles is eliminated on the assumption that it represents mutation rather than crossing over, we obtain totals of 46:30 and 22:29. Alone or combined with previous data these totals show no significant deviations from equality of the two types of doubles.

From the results of the studies reported here and those previously reported (l.c.) we have a considerable body of data bearing on the relative frequencies of two and four strand double exchanges. There is no sound basis for assuming that they occur in any other than the 1:1 ratio expected on the assumption of random crossing over between non-sister strands.

Detected double crossovers in which the rightmost crossover is reciprocal (ba/ab) provide a means of comparing the frequencies of two and three strand double exchanges. Data presented by EMERSON and BEADLE (1933) suggest that three strand doubles occur in the proportions expected

with random crossing over but the data are too limited to establish this conclusion with any degree of certainty. Likewise studies of L. V. MORGAN (1933) on females heterozygous for a closed X chromosome indicate such a relation; here the argument is not as direct as might be desired. The data presented in this paper are the most nearly adequate of any available for testing this point. Following a detected reciprocal rightmost crossover, three classes of double crossover are detected. The type aba/bab comes from a two strand double exchange. The classes bba/bab and aba/aab come from three strand doubles. Four strand doubles are not recovered as double crossovers (see EMERSON and BEADLE for diagrams showing this and the foregoing relations).

TABLE 6

Proportional percentages of genotypes which measure the relative frequencies of two and three strand double exchanges.

CROSSOVER REGIONS	aba / bab	aba / aab	bba / bab
2–3	0.09		
2–4		0.16	
2–5	0.17	0.10	0.45
2–7			0.18
2–8			0.10
3–4	0.07	0.11	0.07
3–5	0.07	0.18	0.26
3–6	0.07	0.18	0.06
3–7	0.21	0.23	0.28
3–8	0.14	0.11	0.07
4–5	0.07		0.13
4–6	0.14	0.09	0.17
4–7		0.17	0.17
4–8	0.07	0.11	0.07
5–6	0.14	0.09	
5–7	0.14	0.11	0.06
5–8	0.22	0.23	0.22
6–8		0.06	
Total	1.60±0.4	1.93±0.4	2.29±0.4

Since the types indicated above all differ phenotypically, it is necessary in comparing their frequencies to use proportional frequencies. These are listed for the three types, for separate regions, in table 6. The totals are 1.60, 1.93, and 2.29 percent for the types as listed. The expected ratio from random crossing over between non-sister strands is 1:1:1. The deviation from this ratio is not statistically significant.

The available evidence from recovered types of double crossovers in attached-X chromosomes can be summarized by saying that the attachment of the strands at the spindle attachment ends does not prejudice the

strands taking part in the crossover nearest to it and that the strands involved in one exchange do not influence those concerned in any other. This is equivalent to saying that if two strands (a and b) cross over at one level then an adjacent crossover between the remaining two strands (a′ and b′) is interfered with to the same extent as is a crossover between the same two strands (a and b); the same relation applies to the other two possibilities at the second level (a and b′ or a′ and b). In other words, the evidence supports the thesis that non-sister strands are involved in crossovers at random.

CROSSOVER VALUES

From the proportional frequencies of the different genotypes as determined in the tests, the crossover values for the different regions can be computed. If this is done by treating the two recovered strands separately, the values obtained are comparable to the standard map values. The number of strands on which these values are based is of course equal to twice the number of tested individuals, that is, 2956. The values obtained in this way together with standard values are given in table 7. It can be

TABLE 7

Crossover frequencies in the eight regions of the attached-X chromosomes. Regions 1 and 2 are computed as twice the observed homozygosis occurring in those regions; other regions are computed from genotypic tests (table 2) treating the two recovered strands separately.

REGIONS	a.f.-cr 1	cr-f 2	f-s 3	s-v 4	v-ct 5	ct-cr 6	cr-ec 7	ec-ac 8
Attached-X	1.6	3.4	10.5	8.7	14.5	7.0	8.2	5.5
Standard	3.5	5.7	13.8	10.0	13.0	6.3	8.2	5.5
Difference	−1.9	−2.3	−3.3	−1.3	+1.5	+0.7	0	0

seen that in all except regions near the spindle attachment the two sets of values show good agreement. The spindle attachment-carnation and carnation-forked values are obtained directly from the homozygosis values for the carnation and forked genes. This is legitimate only on the two assumptions, (1) that crossovers in these regions involve non-sister strands at random with respect to the attachment and that (2) there is no double crossing over within the spindle attachment-forked intervals. The first of these has been shown to be justified; the second is directly tested by the data and is valid. The homozygosis value for a gene, for example, forked, is, in the absence of double crossing over, equal to one-fourth of the total exchange frequency between the spindle attachment and that gene. Standard crossover values represent one-half of the total exchange frequency. Therefore to get a value corresponding to standard crossover values, the homozygosis values are multiplied by two.

The values for attached-X females for the spindle attachment-forked

regions are lower than the standard values for these regions. The value for forked equationals obtained here is 2.51. This agrees fairly well with the value, 2.85, obtained by EMERSON and BEADLE (1933) using the same original stock of attached-X. A third experiment with this stock (BEADLE, unpublished) gave a value of 2.9. Using three stocks of attached-X females (different original occurrences), including the one used here, L. V. MORGAN (1925), ANDERSON (1925), STURTEVANT (1931), and RHOADES (1931) obtained values for forked homozygosis of 5.1, 5.2, 5.1 and 4.9. The explanation of the difference between the two series, which is certainly significant, is not known. Apparently some change which reduces crossing over near the spindle attachment has taken place in the particular stock used here. The higher values obtained by previous workers show that the attachment of the chromosomes does not, in itself, result in any material change in crossing over.

HOMOZYGOSIS

It is apparent that homozygosis for any locus in attached-X chromosomes is a function of crossing over between the spindle attachment and that locus. The observed values for the study reported here are given in table 8. Their relation to map distance from the spindle attachment is given in figure 2.

TABLE 8

Observed and calculated homozygosis values in percentages for genes in attached-X chromosomes.

GENE	OBSERVED	CALCULATED
sc	20.3	18.9
ec	18.4	18.8
cv	18.0	17.8
ct	14.8	16.5
v	11.8	11.1
s	7.0	7.4
f	2.5	2.5
cr	0.8	0.8

From the crossing over detected in recovered strands (genotypic data), the expected homozygosis curve can be calculated. Specific assumptions as to the occurrence of sister strand crossovers and the relation of strands involved in the crossover nearest the spindle attachment and in successive crossovers must be made in order to do this. Such a calculation should serve as a check on the validity of the assumptions that are made. Evidence is available which suggests that sister strand crossovers either do not occur or are not equivalent in interference to non-sister strand crossovers (WEINSTEIN 1932, L. V. MORGAN 1933). The assumption that they do not

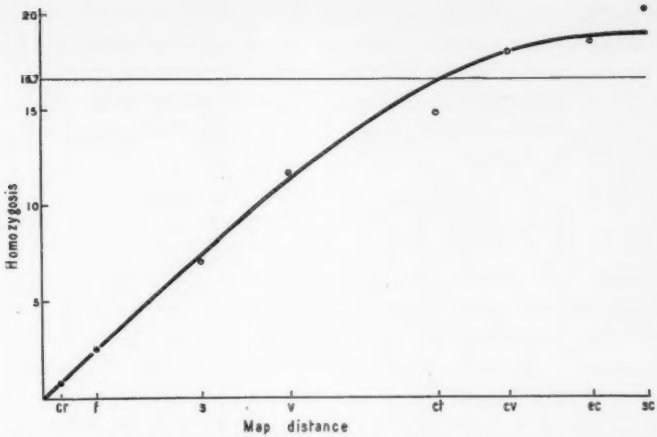

FIGURE 2.—The relation of percentage homozygosis to map distance. Distances on the base line are taken from the crossover values obtained in this experiment (see text). The curve is drawn through the points calculated from the crossover data. The circles represent observed homozygosis values.

occur will be made. The evidence concerning strands involved in multiple exchanges has already been considered and justifies the assumption that the strands involved in crossovers at different levels are independent. From the genotypic data, treating the recovered strands separately, the relative frequencies of strands with 0, 1, 2, 3, and 4 detected crossovers for an interval from the spindle attachment to any marked locus can be calculated. To do this it is necessary to correct for undetectable reciprocal crossovers in regions 1 and 2. The necessary corrected proportional per-

TABLE 9

Proportional percentages of single strands of different crossover types. Corrected for undetected reciprocals in region x.

REGIONS		REGIONS		REGIONS		REGIONS	
0	48.213	x-3	0.135	4-5	0.300	x-3-5	0.075
x*	3.440	x-4	0.190	4-6	0.515	x-3-6	0.010
3	7.658	x-5	0.390	4-7	0.560	x-3-7	0.055
4	6.618	x-6	0.235	4-8	0.370	x-4-5	0.010
5	11.555	x-7	0.290	5-6	0.475	x-4-8	0.020
6	5.550	x-8	0.135	5-7	0.325	x-5-6	0.025
7	5.925	3-4	0.198	5-8	0.725	x-5-8	0.020
8	3.598	3-5	0.550	6-7	0.000	x-6-8	0.010
		3-6	0.352	6-8	0.120	3-7-8	0.035
		3-7	0.885	7-8	0.035		
		3-8	0.493				

* See footnote to table 4.

centages are given in table 9. The corrections are made on the assumption that reciprocal and equational crossovers are equal in frequency (random). From these data the proportions of 0, 1, 2, 3, and 4 exchange tetrads for the interval between the spindle attachment and any designated marked locus can be determined from the following relation between exchanges and detected crossovers:

	Crossovers detected in single strands				
Exchanges	0	1	2	3	4
0	1				
1	1/2	1/2			
2	1/4	1/2	1/4		
3	1/8	3/8	3/8	1/8	
4	1/16	4/16	6/16	4/16	1/16

Having done this, the expected homozygosis value is determined directly from the frequencies with which different numbers of exchanges give homozygosis. These are (for a single allele of a marked locus):

Exchange	Percent homozygosis
0	0
1	25
2	12.5
3	18.75
4	15.625

The two steps can be combined which gives the formula

$$y = 0.5\ S_x - 0.5\ D_x + 1.5\ T_x - 2.5\ Q_x$$

where y is percentage homozygosis for one allele of a gene at locus x and S_x, D_x, T_x, and Q_x are percentages of recovered single strands with 1, 2, 3, and 4 crossovers between the spindle attachment and the locus x. This is the same method of calculation as that used by SAX (1932).

The values calculated by the above method together with the observed values are given in table 8 and shown graphically in figure 2. It can be seen that with the exception of two values, cut and scute, the agreement between observed and calculated values is very good. The observed value for scute is high, that for cut low. The low value can reasonably be ascribed to a disproportionate depression of viability by the cut gene. The value for scute is significantly higher than that calculated from the crossover data. The most reasonable explanation is that the entire calculated curve is depressed somewhat by differential inviability. It is known that flies homozygous for recessive mutants are usually less viable than those of

wild-type. Equational crossovers in proximal regions give, if no other crossover occurs, individuals homozygous for the genes distal to the crossover. However if a second crossover occurs the number of genes homozygous will be reduced. Therefore double crossovers have a certain advantage over single crossovers and there will be a corresponding error in the data. This error will be in such a direction that the calculated curve will be somewhat too low. The high value of scute may then be due to the fact that its effect on viability is less than that of certain of the other genes used.

On the whole the agreement between observed and calculated homozygosis values is good. The agreement constitutes a verification of the assumptions on which the calculations are based, namely that crossing over does not occur between sister strands and is random between non-sister strands. Random crossing over between any two strands including sister strands would give homozygosis values not exceeding 16.7 percent. The observed values obtained by RHOADES (1931) and in the present study clearly exceed this value and therefore confirm the conclusion that sister strand crossovers either do not occur, or are not equivalent to non-sister strand crossovers.

SUMMARY AND CONCLUSIONS

Crossing over was studied over the entire length of the X chromosome in attached-X females of *Drosophila melanogaster*. The genotypic constitutions of 1478 individuals were determined.

The data are in agreement with the assumptions that sister strand crossovers do not occur or at least do not occur independently of non-sister strand crossovers, and that crossing over between non-sister strands involves such strands at random.

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